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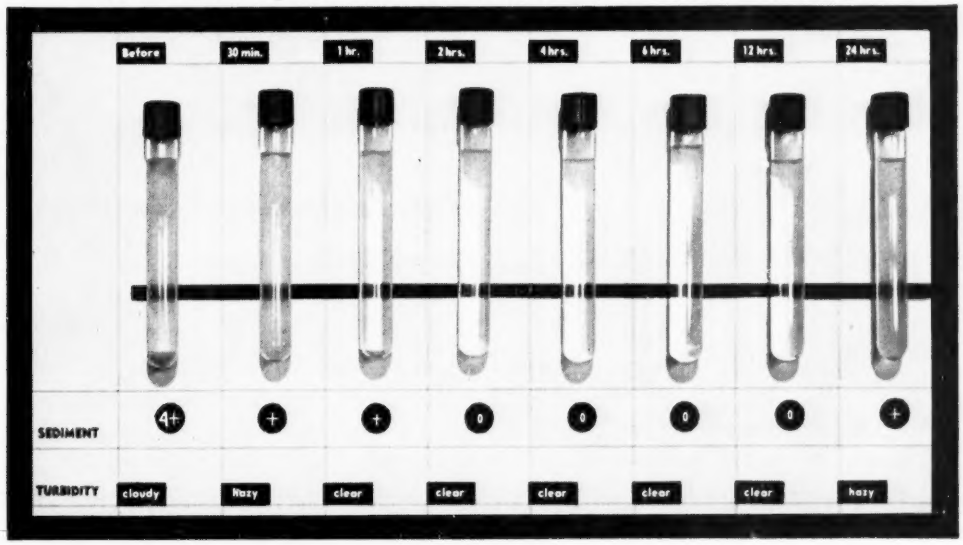

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1. Keyser, L.D.: Arch. Surg. *6*:525-44, 1923; J. Urol. *31*:219-38, 1934; J.A.M.A. *104*:1299-1306, 1935; J. Urol. *50*:169-238, 1943.
2. (a) Butt, A.J.: J. Florida M. A. *37*:711-713 (May) 1951; J. Urol. *67*:450-459 (April) 1952.
(b) Butt, A.J., et al.: New England J. Med. *246*:604-607 (April) 1952; South. M. J. *45*:381-388 (May) 1952; California Med. *76*:123-129 (March) 1952; J.M.A. Georgia *41*:185-190 (May) 1952.
3. Butt, A.J., et al.: J.A.M.A. *150*:1096-1098 (Nov. 15) 1952.

*TRU (turbidity reducing unit) of hyaluronidase is the amount that will in 30 minutes reduce the turbidity produced by 0.2 mg. of hyaluronic acid (mixed with acidified serum) to that produced by 0.1 mg. under standard conditions.

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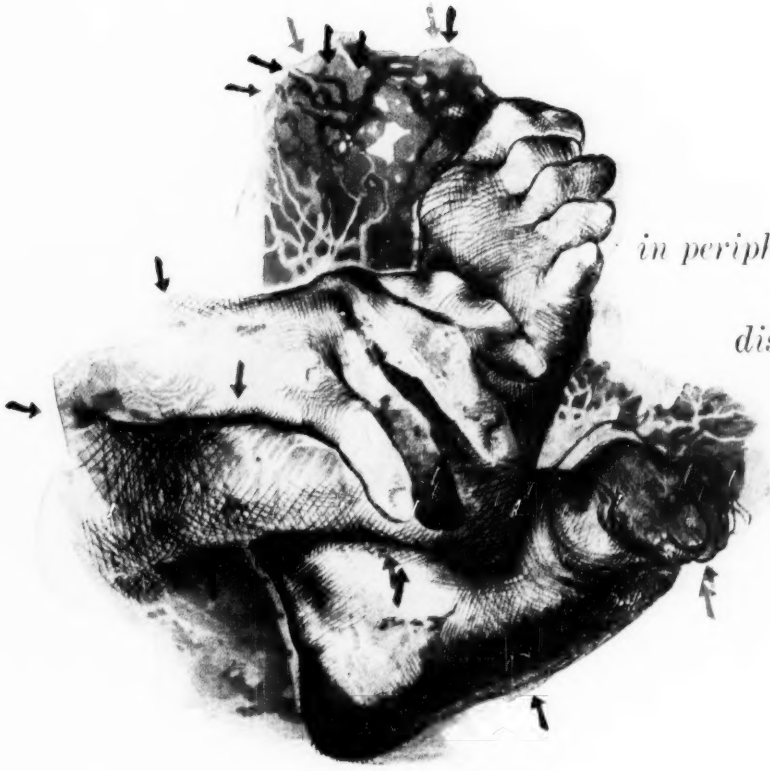

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THE METABOLISM OF GLYCINE-2-C^{14} IN MAN. III. THE URINARY EXCRETION OF C^{14} AND CUMULATIVE RADIATION DOSIMETRY [1]

By NATHANIEL I. BERLIN, BERT M. TOLBERT, AND HELEN C. LEE

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(Submitted for publication May 19, 1952; accepted September 5, 1952)

In previous communications it was reported that following the intravenous administration of 100 microcuries of carbon-14 as methylene labeled glycine (8.0 mg.) 86 per cent of the injected dose was excreted via the lungs as $C^{14}O_2$ (1), and that autopsy data showed there was present a component of elimination of carbon-14 from the tissues having an average half-time of 50 days (2). The present communication is to report the urinary excretion of carbon-14 in patients given 100 microcuries of carbon-14 methylene labeled glycine and to consider the radiation dosage received by these patients.

METHODS

Five patients were given 100 microcuries of glycine-2-C^{14} (8.0 mg.) intravenously; these do not include the four patients whose pulmonary C^{14} excreted has been reported (1). The time and volume of each spontaneous urinary voiding was noted for the first 10 days; thereafter an aliquot of a spontaneous morning voiding was collected once per week. An aliquot (5 or 10 ml.) was dried *in vacuo*, then combusted to carbon dioxide with a chromic-sulfuric acid mixture (3). The carbon dioxide was converted to barium carbonate, the specific activity of which was measured in a proportional counter if greater than 10 dis/min./mg. $BaCO_3$; if less, the sample was reconverted to carbon dioxide and introduced into a 100 cc. ionization chamber operating at atmospheric pressure, a vibrating reed electrometer and recording potentiometer were used to measure the rate of voltage change in the ionization chamber (4, 5).

RESULTS

Figure 1 shows the specific activity of the urinary carbon-14 in patient EP and is typical of the data obtained. Table I summarizes the data for the 5 patients. Figure 2 shows the cumulative urinary carbon-14 excretion in patient EP and is calculated by integration of the data presented in Figure 1 and Table I from the following equation:

$$N = Q\int_0^\infty N_{0.1}e^{-\frac{0.693\,t}{T_{1/2}}} + N_{0.2}e^{-\frac{0.693\,t}{T_{1/2}}} + N_{0.3}e^{-\frac{0.693\,t}{T_{1/2}}}$$

where Q = total C^{14} excreted per day, as gm. $BaCO_3$.

N_0 = extrapolated zero time value for a given component.

$T_{\frac{1}{2}}$ = half-time for a given component.

The amount of total carbon excreted per day was calculated from the amount produced during the first 10 days.

DISCUSSION

Excretion rates

The data indicate that these patients excrete in the urine on the average 11.8 per cent of the carbon-14 administered and that the excretion may be described in terms of three rate components having average half-times of 0.26, 2.5, and 53 days. The urinary carbon-14 radioactivity was followed until it could no longer be measured satisfactorily. Almost complete excretion of carbon-14 via this route would require approximately 200 to 250 days, *i.e.*, five times the half-time of the slowest known component. It should be noted in Figure 1 that during the period from 60 to 85 days the specific activity of the urinary carbon-14 showed a definite increase, occurring at the same time that

TABLE I

The per cent of the administered dose excreted and half-time for each of the three rate processes

Patient	$T_1(T_{\frac{1}{2}})$		$T_2(T_{\frac{1}{2}})$		$T_3(T_{\frac{1}{2}})$		$BaCO_3$ excreted/day	Total per cent excreted
	days	*%*	*days*	*%*	*days*	*%*		
EP	0.10	0.9	1.4	0.9	48	5.7	90.6	7.5
LY	0.37	3.7	2.4	1.5	45	9.0	140.0	14.2
WF	0.17	8.5	1.4	3.3	44	0.6	304.7 (diabetic)	12.4
MM	0.45	5.5	5.0	2.7	84	3.5	82.2	11.7
ESP	0.30	5.7	2.3	1.3	43	6.4	100.9	13.4

[1] These studies were supported by the Atomic Energy Commission.

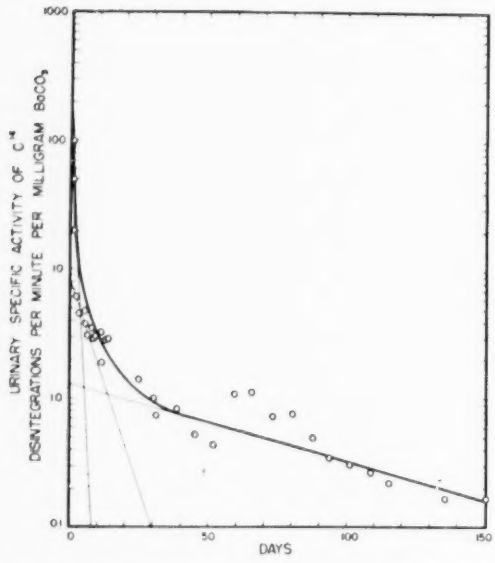

FIG. 1. THE SPECIFIC ACTIVITY OF THE URINARY CARBON IN PATIENT E. P. (IN DIS/MIN./MG. $BaCO_3$)

this patient (with chronic myelogenous leukemia) destroyed the majority of the red cells which had carbon-14 incorporated in their hemoglobin. This probably represents the excretion in the urine of some of the end products of hemoglobin catabolism. It is of interest to note that after four days the specific activity of the urinary carbon gradually becomes much greater than the breath carbon (see Fig. 3, J. Clin. Invest., 1951, **30**, 73). This would indicate a certain independence of the sources of the carbon in these two end-products.

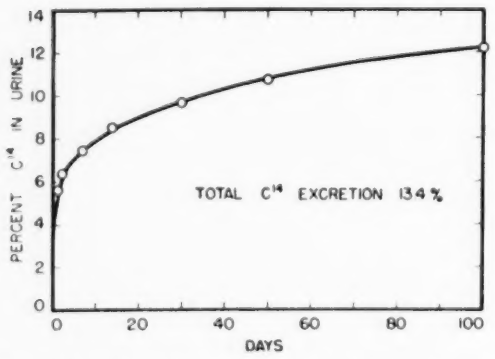

FIG. 2. THE CUMULATIVE URINARY CARBON-14 EXCRETION IN PATIENT E. P.

As has been pointed out with respect to the breath excretion, the biochemical significance of each of these components is not evident. The 53-day urine component is mirrored in the 50-day tissue component both with regard to half-time and to quantity. Thus this 53-day urinary component probably represents the major route of excretion of the 50-day tissue component.

These data now make possible almost complete accountability for the injected carbon-14: 86 per cent is excreted via the lungs; 12 per cent via the urine and 2 per cent is retained for a longer period of time, but must be excreted. No appreciable amount of activity (< 1 per cent) is eliminated in the feces.

The carbon-14 in any given compound will be present largely as the result of synthesis at the time when the precursors have a high specific activity since exchange of carbon atoms in a carbon skeleton is usually not possible without extensive changes to the molecule. Only compounds that are being synthesized will contain carbon-14 and as most compounds are in a steady state there must be a corresponding catabolism and replacement of the carbon-14 by carbon-12 since in the intervening time the precursor specific activity will decrease. It is, however, possible that units such as amino acids can exchange in peptides and perhaps in more complicated proteins. Should such an exchange occur, it would continue and again the carbon-14 concentration must gradually approach natural concentration (6, 7). The autopsy data indicate that the rate of elimination of this small fraction (approximately 2 per cent of the dose) must have a half-time of several hundred days or more. Furthermore, there may be more than one of these very slow components. The route of excretion for this last 1 to 2 per cent will be difficult to determine and will probably involve special preparation of the samples and measuring instruments of great sensitivity.

Calculation of Radiation Dose

The problem of the hazard in the use of carbon-14 in clinical investigation has received considerable attention (8–12). In part the difficulties stem from fundamental lack of knowledge of the effects of ionizing radiation, a fact well recognized by the International Commission on Radiological Pro-

tection (13) (see preface to 1950 recommendations).

However, for the purpose of comparative evaluation, it is of some interest to interpret the results of our studies in terms of radiation dosage. It is acknowledged that several assumptions (detailed below) are necessary in order to make these calculations. The potential error introduced in these assumptions is also estimated.

If it is assumed that this radioactive carbon is uniformly distributed throughout the body, it is possible to make some calculations of the radiation dose delivered to the tissues (14). At zero time the dose rate will be:

$$\text{mrep/day} = \frac{(N)\ (\overline{E\beta})\ (\text{erg/ev})}{(\text{erg/gm./rep})\ (\text{wt. gm.})}$$

N = No. of β/day

$\overline{E\beta}$ = 50,000 ev (average energy of C-14 βs.)

wt. = 70 Kg.

$$\text{ergs/gm./rep} = \frac{83}{1{,}000}$$

mrep/day/100 μc. C-14 = 4.36

In a similar manner, the dose rate at various times may be calculated from the retention of carbon-14. These data are given in Table II and Figure 3. In this figure the "normal" radiation dose is indicated by a horizontal line and is about one-third due to potassium-40 and rubidium-87 (15), one-third to cosmic radiation (16), and one-third from the earth's surface (17). Following the administration of 100 microcuries of carbon-14 the mean radiation dose falls by 40 days to 0.3 mrep/day which is an average "normal" dosage rate.

TABLE II

Cumulative elimination of 100 μc. carbon-14 injected as glycine-2-C^{14}

Time	Microcuries Carbon-14 eliminated			Mrep/day of remaining C^{14}
	Breath	Urine	Total	
0	0	0	0	4.36
1 hr.			4.8	4.15
2 hrs.			10.2	3.91
4 hrs.			18.7	3.54
8 hrs.			27.0	3.18
12 hrs.			32.2	2.95
1 day	40	2	42	2.53
3 days	62	6	68	1.40
5 days	70	6.5	76.5	1.03
10 days	78	8	86	0.61
20 days	80	8	88	0.52
40 days	84	9	93	0.31
60 days	86	10	96	0.175
100 days	86	11	97	0.131
200 days	86	12	98	0.087

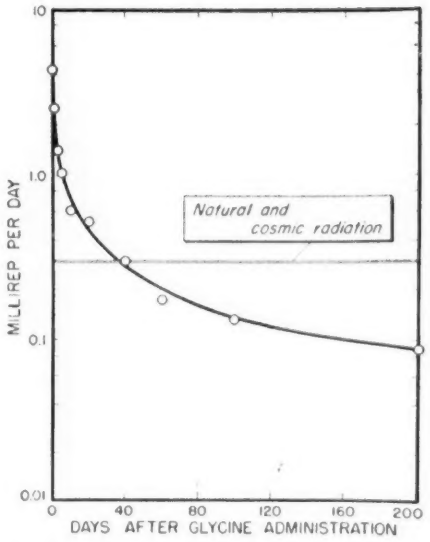

FIG. 3. THE DAILY RADIATION DOSAGE RATE DUE TO CARBON-14

The radiation dosage rate from the naturally occurring radioactive isotopes and cosmic radiation is indicated as being 0.3 mrep/day.

The data listed in Table II were integrated graphically and the cumulative radiation dose determined (see Table III). It must be remembered that these calculations have been made on the assumption of uniform distribution of radioactivity. This is not the true situation and in recommending doses for the use of glycine-2-C^{14} in patients some factor must be included for this nonuniform distribution. This lack of uniformity may be greatest during the first few days following the injection of the carbon-14. However, in

TABLE III

Cumulative carbon-14 radiation dosage for a 70-Kg. man given 100 microcuries glycine-2-C^{14} assuming uniform distribution

Time interval	Dose, mrep
0–1 day	3.18
0–7 days	11.0
0–3 months (13 weeks)	36.9
0–6 months (26 weeks)	45.2

Table III it is seen that the cumulative dose during this period is only about 10 to 20 per cent of the first quarter's total figure, so it is not as critical as inhomogeneities occurring after the first four to five days. The autopsy data (2) and mice data (9) indicate that upper maximum variation from the average tissue activity in this later period is not more than a factor of 2 to 3 so that a factor of 5 (10) for inhomogeneity would probably be a reasonable value. Certainly, at present, the use of 100 μ curies of C^{14} as glycine-2-C^{14} has been sufficient to carry out these studies and yet not exceed the dose permitted by the Atomic Energy Commission for use in patients, with incurable disease and with life expectancy of two years or less.

SUMMARY

1. The urinary excretion of carbon-14 following the intravenous administration of 100 microcuries of carbon-14 as glycine-2-C^{14} may be described in terms of three rate components having half-times of ¼, 2½, and 53 days and amounts to 11.8 per cent of the dose. The 53-day component probably represents the route of excretion of the 50-day tissue component.

2. Complete accountability for the injected carbon-14 is now possible permitting calculation of the dosage rate. For 100 microcuries of glycine-2-C^{14} given to a 70 Kg. man the cumulative radiation doses are: 0 to 1 days, 3.08 mrep; 0 to 7 days, 11.0 mrep; 0 to 3 months, 36.9 mrep.

3. The average internal radiation level from the carbon-14 falls to natural radiation levels in 40 days.

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COMPARISON OF CARDIAC OUTPUT DETERMINED BY THE BALLISTOCARDIOGRAPH (NICKERSON APPARATUS) AND BY THE DIRECT FICK METHOD[1]

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INTRODUCTION

This paper presents a comparison of cardiac output measured by the direct Fick method, with that calculated from the Nickerson-Curtis form of ballistocardiograph (1), and is thus primarily a study of the latter method.

In the form of an apparatus first developed in this country by Starr and his colleagues (2), the ballistocardiograph was designed to measure cardiac output. In normal resting subjects a reasonably satisfactory agreement with cardiac output measured by other techniques was obtained (3, 4). However, the validity of the ballistic record as a measure of cardiac output has been questioned on theoretical grounds (5). When applied experimentally to abnormal circulatory states, moreover, considerable and sometimes wide discrepancies with values obtained by the direct Fick method were found. In traumatic shock, with rapid pulse rates, Cournand and his associates (6) encountered instances in which the ballistic record indicated a markedly increased cardiac output, whereas by the more reliable direct Fick procedure, the actual cardiac output was equally markedly decreased below normal. It was as if the systolic movement of the rapidly beating but relatively empty heart in the presence of a marked reduction of the systemic peripheral resistance produced a ballistic impulse closely simulating that resulting from a large blood ejection.

Extensive further studies, especially by Starr and his colleagues, have led them to the conclusion that the relation between the ballistic record and cardiac output is "a distant one," and that the ballistocardiogram is actually a measure of force rather than of systolic output (7). In spite of the above, the ballistocardiogram continues to be used to measure cardiac output; though sometimes only as an indication of "relative" change from one physiological state to another, in the same individual (8–10). It is thus of some importance to attempt to resolve the question whether such use of this instrument is valid, or not.

In 1944, Nickerson and Curtis reported the design of a critically damped, low frequency ballistocardiograph, in which the natural or periodic oscillation of the Starr apparatus was largely eliminated. Nickerson, Warren, and Brannon (11) later published the results of some measurements comparing cardiac output calculated from this ballistocardiograph with those obtained nearly simultaneously by the direct Fick method. From the first 50 observations in subjects without heart disease, empirical constants were derived for use in the computation of cardiac output. Using these, it was found that 87 per cent of 54 patients with various non-cardiac conditions, had cardiac outputs within 25 per cent of the values obtained with the catheter technique ("direct Fick"). Of 17 patients with heart disease (excluding aortic insufficiency) the ballistic cardiac output was within 25 per cent of the direct Fick in only 60 per cent, but outside this range in 40 per cent of determinations. This rather wide scatter may be compared with the accuracy of the direct Fick method itself, which will be discussed in detail below.

It should be noted that the Warren-Nickerson technique involved voluntary breath-holding during the recording of the ballistocardiogram.

More recently, the Nickerson ballistocardiograph has been used in various studies, but no

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further inquiry has been made of its validity as a method of measuring cardiac output.

As indicated above, the purpose of the present investigation is to analyze further the use of the Nickerson ballistocardiograph as a means of measuring cardiac output, comparing values so obtained, in a considerable variety of clinical material, with those obtained, as nearly simultaneously as possible, by the direct Fick method using cardiac or pulmonary artery catheterization. The experimental work will be presented in three parts: *1*) a study of the effects of breath-holding or voluntary apnea, on the ballistic record; *2*) comparative measurements of ballistocardiograph cardiac output with direct Fick cardiac output in the basal resting state; and finally, *3*) a report of a few experiments which were attempted, to compare ballistic and direct Fick determinations when cardiac output was altered in the same subject, by means of an epinephrine infusion.

PART I

Effects of Respiration and Respiratory Chest Position on the Ballistocardiogram

a. *Apparatus*

The ballistocardiograph was constructed according to the design of Nickerson and varied from it in no essential detail. It had a natural undamped frequency of 1.5 per second. As the weight of the patient varied the spring length was altered in order to maintain the same period. Critical damping was obtained before each study by placing sandbags equivalent to the patient's weight upon the apparatus and adjusting the oil damping system.

b. *Methods*

Ninety-two subjects were studied in all. About 25 per cent of these were normal trained medical students and interns; the remainder were patients selected from the general ward population covering all adult age groups and numerous clinical diagnoses. Not all of the subjects were in a basal state, but all lay on the ballistocardiograph for a varying period, usually about 30 minutes, until the blood pressure and the pulse rate had become stable. Several records were taken on each subject during quiet respiration and voluntary apnea. The breath was held in midposition.

A number of records were also taken on trained normal subjects during quiet breathing and with the breath held in various phases of the respiratory cycle and consequently a varying volume of air in the lungs and varying point of fixation of the diaphragm. The subjects breathed into a spirometer simultaneously with the recording of the ballistocardiogram. In this manner the exact position in which the breath was held and the actual volume of air in the lungs at the time of the output measurement could be graphically recorded.

Cardiac output determinations were calculated from the Nickerson formula,

$$SV = 5.02 \frac{F \times P}{T \times L} (1),$$

where: SV = stroke volume in cubic centimeters

5.02 = the empirical constant determined by Nickerson to provide the best fit between the ballistic results and the direct Fick data

F = the force required to displace the apparatus the distance between the peak of the first major footward deflection (I) and the first major headward deflection (J). (This is derived from a simple ratio using a 50-gram weight to calibrate the bed.)

P = the square root of one-half of the sum of the systolic and diastolic blood pressures

T = the time, in seconds, from the peak of the I-wave to the peak of the J-wave

L = the length of the patient in centimeters.

c. *Results*

The general average of ballistocardiographic cardiac output determinations during quiet breathing was 5.28 liters per minute as compared with 5.57 liters per minute with the breath held in midposition. The difference of 0.29 liters per minute or 6 per cent is not significant. However, a marked variation was observed between individual records.

Figure 1 is a frequency distribution curve showing that only 43 of the 92 cases (or 47 per cent) fall into the range of ± 10 per cent of exact agreement; only 62 (or 67 per cent) fall into the range

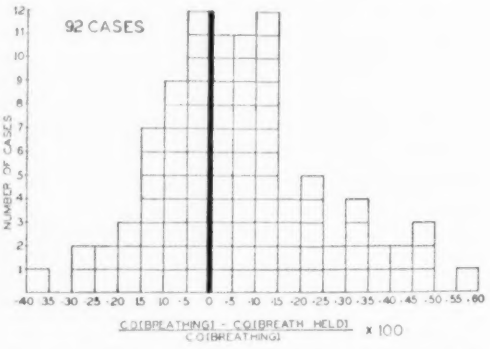
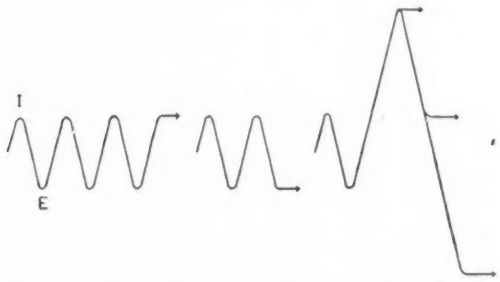


FIG. 1. EFFECTS OF BREATH-HOLDING VS. QUIET RESPIRATION ON CARDIAC OUTPUT AS CALCULATED FROM THE BALLISTOCARDIOGRAM

of ± 15 per cent. In the remainder, 33 per cent of the total, the variation exceeded 15 per cent, with an overall range of − 40 per cent to + 60 per cent.

Figure 2 is a diagrammatic representation of the results obtained when a trained subject breathed into a spirometer, simultaneously with the recording of the ballistocardiogram. This diagram was compiled from the data of one individual, but variation of a similar degree was observed in all of the patients studied. It is seen that in the case illustrated, with a fairly constant heart



HR	65	65	60	65	63	65
SV	130	145	139	183	160	149
CO	8.5	9.5	8.4	11.9	10.1	9.7

FIG. 2. EFFECTS OF RESPIRATORY POSITION OF THE CHEST ON CARDIAC OUTPUT, AS CALCULATED FROM THE BALLISTOCARDIOGRAM, IN ONE NORMAL SUBJECT

Curve gives a schematic representation of the chest position at the time each ballistocardiogram was taken, with the figures (indicated below) of the corresponding cardiac output volumes. HR = heart rate per minute. SV = stroke volume in cc. C.O. = cardiac output in liters per minute. From left to right the figures represent: *1*) quiet breathing, *2*) end of normal inspiration, *3*) end of normal expiration, *4*) deep inspiration, *5*) mid-position after deep inspiration, *6*) deep expiration.

rate, both the stroke volume and the cardiac output varied by as much as 40 per cent, according to the position in which the breath was held. In this particular case the lowest values for cardiac output were found with the breath held following a normal expiration, the highest at the point of maximal inspiration. In six other patients similarly studied, there was a comparable degree of variation in the cardiac output values obtained with the breath held in different positions. However, the maximum value was not constantly found at the height of inspiration.

d. *Comments*

It has long been known that any change from normal resting respiration will upset the steady state of the circulation. The experiments above described are few, but indicate that voluntary breath-holding causes somewhat unpredictable but at times large changes in the ballistic record. This problem has also been studied by Starr and Friedland (12). Although the general average of the calculated cardiac outputs while breathing is the same as the average calculated from records obtained during apnea, this finding is not of primary interest, since it is the validity of the individual cardiac output measurement that we are concerned with here. When the position of apnea was recorded and the actual volume of air in the lungs measured, the influence on the cardiac output as recorded by the ballistocardiograph appeared to depend upon the volume of air in the lungs and the position of the diaphragm. In individual cases the cardiac output as measured by the ballistocardiograph was found to vary by more than 3 liters dependent entirely upon the actual position in which the breath was held. These findings would tend to indicate that the ballistic records taken during voluntary apnea are unreliable and offer no standard basis upon which cardiac output determinations may be compared.

Since records taken with the breath held seemed unreliable, our attention became focused upon those taken during quiet breathing. Here also considerable difficulty was encountered. The natural period of the bed is low and of such a nature that the type of respiration often interferes with the recording of a readable ballistic pattern. In general, the more rapid and vigorous the respiratory effort, the greater the degree of interference and therefore the more limited the use of the low frequency ballistocardiograph. In order to obtain a satisfactory record during breathing, a moderate degree of cooperation of the subject is required, in keeping the respirations quiet and even. This was found to be no problem in young normal subjects, but with the general ward population at Bellevue Hospital nearly 40 per cent of the patients were found to be unsatisfactory subjects. Patients with pulmonary disease and patients in congestive heart failure comprise a group where dyspnea is frequently marked. In

TABLE I

Comparison of cardiac output by direct Fick method (cardiac catheterization) and by Nickerson ballistocardiograph

Subject	Sex	Age	Height	Weight	O_2 Cons.	O_2 A−V diff.	Cardiac output Fick	Pressures Ps	Pressures Pd	Heart rate	Cardiac output Ballisto-cardiograph	Diagnosis
		yrs.	*in.*	*lbs.*	*cc./min.*	*cc./100 cc.*	*l./min.*	*mm.Hg.*			*l./min.*	
A. C.	F	33	60½	103	157	2.9	5.42	96	58	62	2.53	Oxyuriasis
J. B.	M	62	68	130	182	5.0	3.64	126	72	77	4.10	Ca. of cardia
P. D.	M	61	69	134	237	4.4	5.39	122	68	60	2.45	Pulmonary Tbc., IIB
J. N. (1)	M	42	68½	152	254	3.8	6.68	108	70	58	4.35	Bronchiectasis
(2)					253	3.7	6.84	112	70	57	3.15	
W. P. (1)	M	39	72	163	253	3.1	8.16	102	70	62	4.30	Nephritis
(2)					255	3.2	7.96	106	70	62	4.09	
H. W.	M	39	67	133	229	4.7	4.87	130	95	61	4.30	Gastroenteritis
J. M. (1)	M	56	68½	125	254	2.9	8.76	128	78	79	4.80	Tbc. and pernicious
(2)					239	3.0	7.97	135	80	74	5.20	anemia
C. L. (1)	M	50	69½	139	254	3.6	7.06	110	70	57	3.30	Chronic alcoholism
(2)					224	3.3	6.79	112	72	56	2.85	
N. G. (1)	M	35	69	164	207	3.2	6.46	112	72	60	4.80	Peptic ulcer
(2)					213	3.0	7.10	116	73	66	5.30	
J. P. (1)	M	52	69½	136	232	4.0	5.80	125	85	78	4.90	Tbc.
(2)					215	3.1	6.62	130	80	71	4.70	
R. R. (1)	M	45	63½	133	255	4.8	5.32	125	85	66	4.15	Alcoholism
(2)					236	4.5	5.24	120	75	65	3.90	
W. G. (1)	M	54	62½	119	307	4.2	5.60	120	88	80	3.90	Diverticulosis
(2)					301	4.4	5.20	130	90	80	3.20	
D. B.	M	45	66	148	248	2.8	8.86	104	83	74	4.55	Pneumonia
R. N. (1)	M	50	65½	115	178	3.8	4.69	116	74	74	4.88	Pneumonia
(2)					183	3.1	5.90	116	74	70	5.04	
C. B. (1)	M	53	66	135	237	3.9	6.09	98	70	68	3.97	Bronchiectasis
(2)					220	3.6	6.12	105	75	64	4.40	
S. G. (1)	M	48	68	143	295	3.2	9.22	130	88	112	7.73	Atypical pneumonia
(2)					287	3.1	9.25	137	88	110	8.20	
A. A. (1)	M	46	67½	134	261	3.0	8.70	122	78	72	4.68	Infectious hepatitis
(2)					260	3.6	7.22	128	78	68	3.76	
E. H.	M	54	66½	130	225	3.1	7.25	102	68	80	4.47	Pneumonia
J. N. (1)	M	62	73	174	222	5.0	4.44	160	105	66	3.80	Hypertensive cardio-
(2)					226	5.3	4.24	160	108	65	3.55	vascular disease
T. M. (1)	M	37	66	158	248	3.4	7.29	108	70	68	3.95	Peptic ulcer
(2)					245	3.9	6.28	118	70	67	4.10	
B. P.	M	41	63½	156	254	3.9	6.52	240	165	97	5.48	Hypertensive cardio-vascular disease
C. G. (1)	M	59	68½	140½	202	3.7	5.46	110	76	61	2.04	Nephritis
(2)					207	3.7	5.59	110	75	61	2.01	
F. S. (1)	M	27	69½	142	253	3.4	7.44	110	68	60	3.57	Pneumonia
(2)					236	3.4	6.94	112	62	59	3.79	
J. S.	M	46	67	130	192	4.1	4.68	92	64	70	4.10	Pneumonia
J. B. (1)	M	60	65	110	188	3.8	4.95	100	72	80	6.70	Cor pulmonale, ASHD
(2)					183	3.3	5.54	100	72	80	6.34	
C. D.	M	31	68½	176	269	3.5	7.68	110	78	63	5.60	Nasopharyngitis
T. W.	M	41	68	134	225	3.5	6.43	126	78	81	5.15	Lymphogranuloma venereum
B. P. (1)	M	41	63½	156	247	3.8	6.50	190	110	92	6.50	Hypertensive cardio-
(2)					238	3.8	6.27	190	110	93	6.22	vascular disease
C. M.	M	40	64	128	236	4.0	5.90	125	70	76	5.10	Peptic ulcer
C. R. (1)	M	49	69½	123	191	4.4	4.34	190	110	72	4.50	Hypertensive cardio-
(2)					214	4.8	4.46	190	110	70	4.08	vascular disease
C. R.	M	49	69½	123	228	5.0	4.56	220	125	82	4.85	Hypertensive cardio-vascular disease
A. Y.	M	37	64¼	96½	246	3.4	7.24	120	75	88	6.60	Cor pulmonale
M. W.	F	40	62	142	245	4.2	5.84	180	90	86	4.95	Hypertensive cardio-vascular disease
J. O. (1)	M	22	70	132	220	4.4	5.00	108	70	72	5.30	La grippe
(2)					226	4.5	5.02	108	80	66	4.60	
W. H.	M	50	68	153	236	5.0	4.72	220	125	60	4.10	Hypertensive cardio-vascular disease
C. A. (1)	M	22	64½	122	247	4.1	6.03	115	80	91	7.40	Pulmonary Tbc., minimal
(2)					244	4.0	6.10	115	80	85	7.30	
J. H. (1)	M	21	75	190	322	3.5	9.20	124	80	65	7.90	Hepatitis
(2)					316	3.9	8.11	120	80	63	6.82	
H. W.	M	38	69	141	267	6.0	4.45	215	150	96	4.60	Hypertensive cardio-vascular disease
M. C. (1)	M	45	68	141	246	3.5	7.04	202	105	59	1.68	Hypertensive cardio-
(2)					220	3.6	6.12	210	105	56	1.88	vascular disease

the presence of dyspnea it was usually impossible to secure a calculable ballistic record.

PART II

Comparison of Cardiac Output Determinations by the Ballistocardiographic and the Fick Methods

a. *Methods*

The subjects studied were selected from the general hospital population. They were chosen on the basis of cooperation, calculability of the ballistic record, and, except in two instances, the absence of valvular heart disease. All were in a basal state in the morning. These patients suffered from a variety of clinical conditions, as indicated in Table I. However, none were febrile and all of those suffering from an acute disease were far along in the convalescent state.

An intracardiac catheter was inserted into the right heart under direct fluoroscopic vision. In the earlier cases it was placed in the right auricle just in front of the tricuspid valve; in the majority of cases, however, in the main pulmonary artery. The subject was then transferred by means of a stretcher to the ballistocardiograph. After a period of stabilization, cardiac output measurements were made by both methods as nearly simultaneously as possible. For the Fick determinations, expired air was collected over a 2-minute period. Thirty seconds after the beginning of the collection of expired air, arterial and mixed venous blood samples were drawn slowly and simultaneously over a 1-minute period. Blood analyses were performed according to the method of Van Slyke, gas analyses according to Scholander. Duplicate determinations were made on each sample.

The ballistocardiograms used for comparison with the direct Fick values were taken during quiet breathing. Since the ballistic record varies from inspiration to expiration, the values from all heart beats through at least two respiratory cycles were calculated individually and then averaged, to give the cardiac output figure.

In all, 64 determinations were made on 40 individuals. All ballistocardiographic cardiac outputs were calculated from records taken during quiet breathing. Again all calculations were made according to the formula

$$SV = 5.02 \frac{F \times P}{T \times L},$$

as already described.

b. *Validity of the direct Fick method for measuring cardiac output*

The validity of the Fick method as a physiological measurement of cardiac output has recently been carefully studied by Seely, Nerlich, and Gregg (13). These authors, using anesthetized dogs, made serial determinations of cardiac output using both the Fick and the rotameter methods. The agreement was excellent. The average variation was ± 5 per cent. When the amount of blood flow was experimentally increased by the infusion of whole blood or epinephrine, the close relationship between the two methods was maintained. The authors conclude that "when the Fick procedure was compared to a direct method of high accuracy, there was excellent agreement in a variety of hemodynamic states."

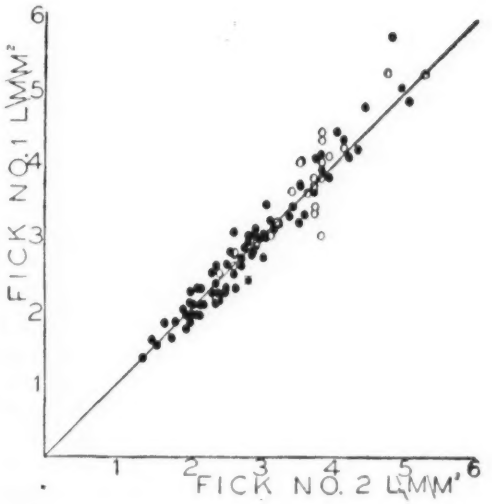


FIG. 3. SCATTER DIAGRAM OF CARDIC INDICES AS MEASURED BY THE DIRECT FICK METHOD ON TWO SUCCESSIVE DETERMINATIONS

Open circles represent cases included in the present study.

There remains the question of the accuracy of the Fick procedure in the hands of any individual investigator or laboratory. In an effort to evaluate the Fick method and to establish the degree to which it can be reduplicated, we have recently analyzed the data from the Cardiopulmonary Laboratory of the Columbia University Division at Bellevue Hospital.

In all, 100 cases were found where successive Fick determinations had been made on the same individual under the same physiological conditions during the past four years. Of these 100 cases, 22 are included in the present study. The cases were taken consecutively and represent all subjects studied in this Laboratory where successive determinations were made at rest, under

basal conditions, and during a steady state of respiration and circulation. These data were accumulated by three different catheterization teams. The subjects include all ranges of cardiac output levels with varied clinical conditions. There are many normal individuals and many with heart disease and far advanced congestive heart failure.

The variation of successive Fick determinations in these 100 cases was found to be 0 to 5 per cent in 52 cases, 6 to 10 per cent in 31 cases, and 11 to 15 per cent in 15 cases. There were only two instances where successive measurements differed by more than 15 per cent. Thus in 83 per cent of the cases studied, the variation was 10 per cent or less. The average variation was 6.6 per cent. The mean cardiac index in one series of duplicates was 2.97 liters per minute per square meter, as compared with a mean of 2.94 liters per minute per square meter in the other.

The average variation of the 22 cases included in the present study was identical to the above. The frequency distribution curve of these 22 was likewise similar to that of the entire group.

A statistical analysis of the data obtained from the successive determinations revealed that *1*) there was a correlation coefficient of 0.975, and *2*) the standard deviation of a single cardiac index determination was 0.17 liters per minute per square meter of body surface.

It was further calculated from the standard deviation that any two independent observations on the same patient may with 95 per cent confidence vary one from the other by no more than 0.48 liters per minute per square meter. In about 70 per cent of the duplicates the difference will not exceed 0.24 liters per minute per square meter. The scatter of these results is plotted in Figure 3.

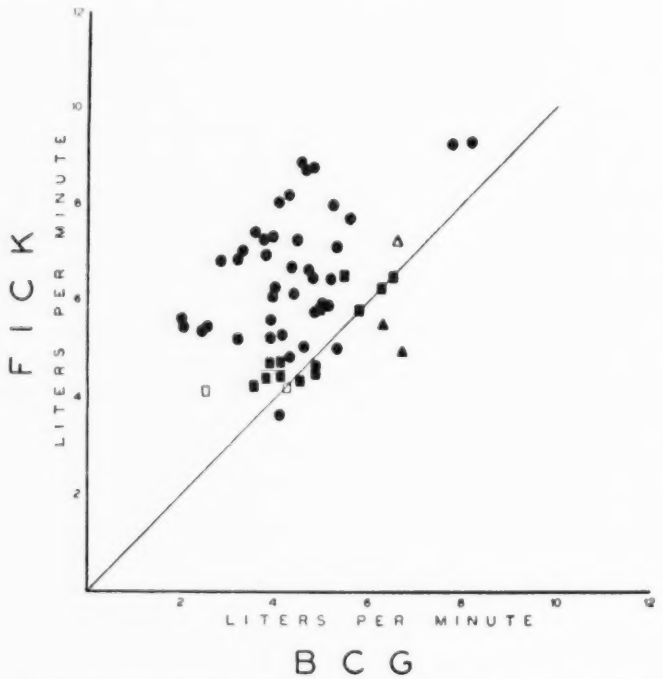


FIG. 4. SCATTER DIAGRAM OF CARDIAC OUTPUT CALCULATED FROM BALLISTOCARDIOGRAM, AS COMPARED WITH CARDIAC OUTPUT BY THE DIRECT FICK METHOD. CALCULATIONS ACCORDING TO NICKERSON FORMULA

Solid circles = patients with no heart disease. Solid squares = hypertensive vascular disease. Open squares = mitral stenosis. Triangles = cor pulmonale. These cases, and a few additional cases as well, are presented also in Table I.

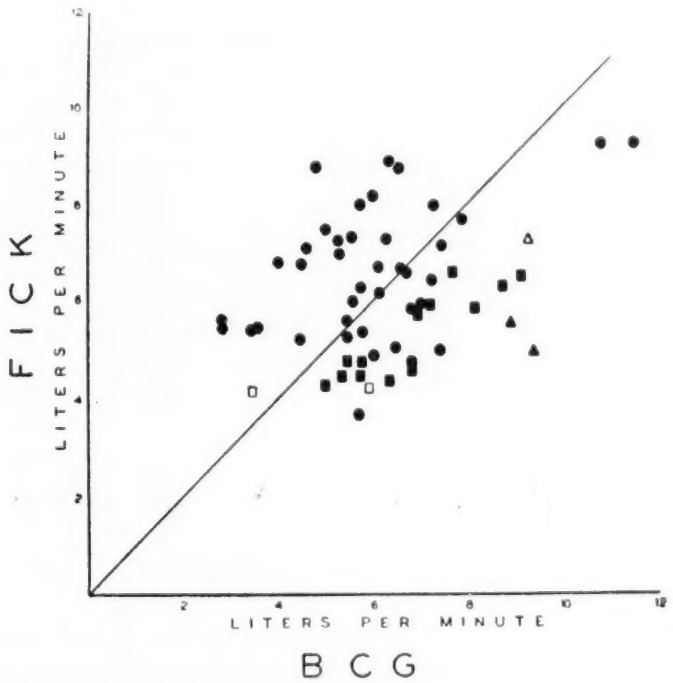


FIG. 5. THE SAME AS FIGURE 4, EXCEPT THAT ALL BALLISTOCARDIOGRAM FIGURES HAVE BEEN MULTIPLIED BY THE FACTOR 1.4. SEE TEXT

c. *Results*

In the group of patients studied in the basal state the average cardiac output as calculated by the direct Fick procedure was 6.23 liters per minute, as compared with an average ballistocardiographic output of 4.49 liters per minute. The average cardiac index by the Fick method was 3.6; by the ballistocardiograph, 2.6. The correlation coefficient was 0.256, the standard error $\pm$ 0.128, indicating no significant correlation. Figure 4 is a scatter graph of these results about the line of unity.

In Figure 5, the generally higher values of the cardiac outputs by the Fick method over those by the ballistocardiographic method have been corrected by applying to the latter an arbitrary correction factor of 1.4, *i.e.* $\frac{3.6}{2.6}$ ratio. The line of unity runs through the center of the scatter. Even with this correction, however, marked discrepancies in a large proportion of instances still persist.

d. *Comments*

In the series reported by Nickerson, Warren, and Brannon (11), as already noted, the agreement in cardiac output between ballistocardiographic and direct Fick measurements was not very good, especially in the cases with heart disease. The normal figures were of course grouped evenly around a mean, since it was from these figures that the constants of the formula were derived. In our series, the normal subjects gave a quite different mean value of cardiac output as determined by ballistocardiogram. The scatter of our values, compared with the direct Fick measurements, was somewhat greater than those of Nickerson, Warren, and Brannon, perhaps because our patients were suffering from a wider variety and severity of cardiac, pulmonary, and other diseases, thus bringing more variable influences to bear upon the ballistic record.

It is known (6) that large differences in pulse rate will increase the error in cardiac output cal-

TABLE II

Cardiac output data on subjects given intravenous epinephrine

Subject	Condition of study	O_2 Cons.	O_2 Cons.	O_2 A−V diff.	Cardiac output Fick	Blood pressure	Heart rate	Cardiac output B.C.G.
		cc./min.	*cc./min./ sq.m.B.S.*	*cc./100 cc.*	*l./min.*	*mm.Hg*		*l./min.*
J. S.	Control	192	114	4.1	4.68	92/64	70	4.10
	Epinephrine i.v. 10 min.	218	130	2.7	8.07	95/70	72	5.20
	Epinephrine i.v. 20 min.	202	119	3.5	5.77	100/65	74	6.40
	Post epinephrine 20 min.	196	117	4.4	4.45	102/70	72	4.00
C. D.	Control	269	138	3.5	7.68	110/78	63	5.60
	Epinephrine i.v. 10 min.	272	139	3.5	7.77	118/82	70	6.48
	Epinephrine i.v. 20 min.	291	149	3.8	7.66	118/82	70	6.90
	Post epinephrine 20 min.	294	151	3.8	7.74	118/82	62	4.65
T. W.	Control	225	130	3.5	6.43	120/88	80	5.15
	Epinephrine i.v. 10 min.	268	155	2.4	11.20	132/68	91	7.98
	Epinephrine i.v. 20 min.	321	185	2.4	13.40	138/68	99	7.62
	Post epinephrine 20 min.	261	151	3.3	7.91	130/80	86	5.76
F. J.	Control	—	—	—	—	130/55	76	7.35
	Epinephrine i.v. 10 min.	266	147	1.6	16.60	160/40	94	18.40
	Epinephrine i.v. 20 min.	317	175	2.0	15.85	160/40	92	17.80
	Post epinephrine 20 min.	285	157	3.2	8.92	130/65	84	7.99
C. M.	Control	236	147	4.0	5.90	125/70	76	5.10
	Epinephrine i.v. 10 min.	238	148	2.6	9.16	128/72	92	10.40

culated from the ballistocardiogram. It would seem from our data that another factor causing error, at least in the use of the Nickerson formula, may be the blood pressure. Figures 4 and 5 indicate that all cardiac outputs in hypertensive patients lie along the lower edge of the group, *i.e.*, ballistic cardiac outputs are relatively high. Examination of the individual cases shows that this is entirely due to the factor P in the formula, since the ballistic complexes themselves are small.

With so poor a correlation between ballistocardiographic and direct Fick cardiac outputs, the only conclusion to be reached is that this type of ballistocardiograph does not provide a means of measuring cardiac output, in a group of patients such as those represented in this series, even though a new empirical constant is introduced in the formula.

PART III

Comparison of Ballistocardiographic and Direct Fick Methods, when Cardiac Output is Altered, in the Same Subject

There remains the question whether the ballistocardiograph can be used to indicate relative cardiac output changes in the same individual, for example as a result of drug action. An infusion of epinephrine to increase the cardiac output has been used to put this question to the test.

a. *Method*

A control cardiac output determination was made by each method. A slow infusion of epinephrine was then started, the rate of flow of which was adjusted so that the patient received either 0.10 μ or 0.15 μ per Kg. of body weight per minute. Repeat measurements were made at 10 minutes and 20 minutes. The infusion was then terminated and an additional cardiac output determination by each method was made after a 20-minute interval.

Five patients were so studied. It was a long and difficult procedure, and while every effort was made to establish a steady state of the circulation during the infusion, this was not completely successful in every instance. Obviously the validity of the direct Fick procedure, and to some extent of the ballistocardiographic method also, depends upon the establishment of a new "steady state" of the circulation at a different level during the time the measurements are taken.

b. *Results*

The results are given in Table II. A relatively steady state, so far at least as oxygen consumptions are concerned, existed in three of the five cases, J. S., C. D., and C. M.; in the other two, the oxygen consumption increased during a part of the procedure.

In one case, C. D., the cardiac output did not

change as measured by the direct Fick method, whereas an increase was calculated on the basis of the ballistocardiogram. In the others, increases were measured by both methods; in two instances the amounts of the increase were roughly comparable; in the other two they were not.

c. *Comments*

It is suggested from these limited data that cardiac output changes produced by epinephrine infusion are not recorded equally or proportionately by ballistocardiograph as compared with direct Fick measurement. Further study would be needed to define these discrepancies more exactly.

CONCLUSION

The general conclusion to which this study leads is that the ballistocardiographic measurement of cardiac output gives such erratic results, with so many and such unpredictable errors from case to case, that it should no longer be used in any way whatever as an indication of this function. Earlier studies with the Starr apparatus have pointed to the same conclusion (6, 7). Conversely, it would seem more logical to use this instrument solely as a qualitative recording of the ballistic impulse as such, analyzing the form of the records empirically, for such correlations as can be found with clinical signs, symptoms, and prognosis (14, 15).

SUMMARY

1. A comparison has been made of cardiac output measured by the direct Fick (cardiac catheterization) technique, and by the use of a Nickerson critically damped ballistocardiograph, using Nickerson's formula. Sixty-four comparative determinations were made in 40 individuals, both normal subjects and patients with a variety of clinical conditions.

2. It was found that voluntary apnea or breath-holding altered the ballistocardiographic record in an irregular and unpredictable manner. Ballistocardiograms were therefore taken during quiet breathing. In about 40 per cent of hospital patients, especially those with cardiac or pulmonary disease, the records were so distorted as to be unreadable, due largely to respiratory overactivity.

3. Comparison of cardiac output calculated from ballistocardiograph records and measured by the direct Fick method showed a wide scatter and poor correlation, both under basal metabolic conditions and during an intravenous infusion of epinephrine.

4. It is concluded that this type of ballistocardiograph does not provide a method of measuring cardiac output.

ACKNOWLEDGMENT

The authors are indebted to Dr. Donald Mainland of New York University for the statistical analysis of the Fick data.

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ISOTOPIC STUDIES OF POTASSIUM METABOLISM IN DIABETES [1, 2]

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It is well recognized that deficits of cellular potassium and hypokalemia may develop in diabetic patients under treatment for acidosis and coma (1, 2). No data are available, however, concerning potassium metabolism in less severe stages of diabetes, in the presence of complications, or in diabetics with intercurrent disease. The availability of the artificial radioactive isotope, K^{42}, has made possible a direct measurement of the "exchangeable body content" of potassium (3). The following study was performed in an attempt to measure and evaluate the exchangeable potassium content (Ke) in diabetic patients at various stages of regulation and during treatment for various complications; and to determine the effect of orally administered potassium salts upon the body content of potassium.

MATERIAL

The subjects were 42 unselected diabetic patients—22 females and 20 males between the ages of 18 and 78 years—who were hospitalized for regulation of the diabetes, for treatment of complications, or for causes unrelated to the diabetic condition. The cases varied widely in severity, duration and adequacy of control. One subject (case 9) was in acidosis and coma. All but two (cases 29 and 30) required insulin. Pertinent information concerning the clinical status of these 42 patients is given in Tables I and II.

The shipments of K^{42} were received on Monday and were treated in the manner described by Corsa and his co-workers (3). One and a half milliequivalents of potassium chloride solution, containing 100 microcuries of K^{42}, was administered intravenously to those subjects injected on Tuesday. Three milliequivalents of potassium containing 100 microcuries of K^{42} were injected into those subjects who were given the material on Wednesday.

METHODS

The initial determination of the exchangeable potassium content (Ke-1) was performed as soon as possible after the patient's admission to the hospital. Whenever possible, three serial determinations were performed at intervals of six to eight days. In all instances the usual routines of diabetic therapy, including diet, insulin and vitamin supplements, were followed.

In some cases, in order to determine the effect of an oral supplement on the body potassium content, potassium chloride, 1 Gm. thrice daily (a total of 40 milliequivalents of potassium daily), was given for one week after the initial determination of Ke (Ke-1) was made. At the end of the week a second determination (Ke-2) was made, and whenever possible a third determination (Ke-3) was made one week later.

The technique used for the Ke determinations was as follows: The subject was given an intravenous injection of radioactive potassium by calibrated syringe between 7:30 and 9:00 a.m. All urine specimens until 7:00 a.m. on the following day were collected and pooled, and this collection of urine was measured for the excretion of K^{42}. Specific activity of the urine was determined on two spot samples collected at 8 and 9 a.m. on the day after injection.

Preliminary studies confirmed the observation of Corsa and his associates (3) that the specific activity of K^{42} in the urine reached an equilibrium within 24 hours. The mean difference in specific activity between the two spot specimens, when expressed as percentage of the mean Ke, was 6.52 ± 6.65 per cent. In normal individuals the administration of supplemental oral potassium, 3 Gm. daily for six days, resulted in a mean increase in Ke of 8.5 per cent; the maximum increase was 20.2 per cent in one male subject (4).

Measurement of radioactivity

The activity of the urine specimens was determined with a dipping tube and a scaling circuit. A total of 10,240 counts was made on all samples. All counting rates were at least ten times background, and were usually in the range of 500 to 3,000 per minute. At these counting rates, no correction for coincidence loss was necessary. All determinations were corrected for decay.

[1] This work was supported in part by a grant-in-aid from the American Heart Association and in part by the U. S. Atomic Energy Commission under a contract with the Bowman Gray School of Medicine.

[2] The K^{42} was supplied by the National Laboratory, Oak Ridge, Tenn., on allocation from the U. S. Atomic Energy Commission.

[3] Research Fellow of the American Heart Association sponsored by the 20-30 International Rheumatic Fever Foundation.

Chemical determinations

The total inorganic potassium concentration in the urine was determined by flame photometry, using a method which has been described previously (5).

Calculation of exchangeable potassium

The following formula was used to calculate the value for the exchangeable potassium content of the body:

$$Ke = \frac{Ki^{42} - Ko^{42}}{Ku^{42}/Ku^{39}}$$

Ke = quantity of exchangeable potassium in milliequivalents.

Ki^{42} = quantity of radiopotassium administered (arbitrary units).

Ko^{42} = quantity of radiopotassium excreted in the urine in the pooled specimen.

Ku^{42} = concentration of radiopotassium in the spot samples.

Ku^{39} = concentration of inorganic potassium in the spot samples.

Ku^{42}/Ku^{39} = mean specific activity of the two spot specimens.

RESULTS

A total of 72 determinations of Ke were made in the 42 subjects. In 22 individuals only a single determination was obtained. Serial determinations were made in 5 subjects who did not receive an oral supplement of potassium. In the remaining 15 cases supplemental potassium was given and two or more determinations of Ke were made.

TABLE I

Exchangeable potassium content in diabetic males

Patient	Age	Weight	Ke	Change in Ke	Duration of diabetes	State of disease	Complications	Incidental diseases
		Kg.	*(mEq.)*	%				
					Serial determinations with KCl supplement			
1	34	60.9	1923*		11 yrs.	Out of regulation 3 mos.	Renal insufficiency and hypertension	
		58.2	2407†	+25.2				
		58.2	2485‡	+29.2				
2	40	52.3	1553*					Duodenal ulcer, 3 mos. Developed pyloric obstruction between Ke-2 and 3. Clinical potassium deficiency with Ke-3
		48.3	1873†	+20.6				
		47.5	1463‡	− 5.8	4 yrs.	Poor control		
		47.3	1985§	+27.8				
3	68	76.1	2101*		3 mos.	50 lbs. wt. loss in 2 yrs.		
		73.4	2404†	+14.4				
4	71	61.1	1544*		3.5 yrs.	50 lbs. wt. loss in 1 yr. Diabetes easily controlled	Gangrene of toe, 1 yr. Supracondylar amputation 4 days before Ke-3	
		61.7	1524†	− 1.3				
		60.9	1252‡	−18.9				
5	66	67.7	2441*					Benign prostatic hypertrophy. Prostatectomy between Ke-1 and Ke-2
		65.9	2243†	− 8.1	2 wks.	Very mild		
6	46	66.4	2146*		11 yrs.	Good control	Gangrene of toes. Supracondylar amputation between Ke-1 and Ke-2	
		66.4	2158†	+ .6				
7	77	57.3	1544*		Diagnosed during hospitalization	Regulated without difficulty		Prostatic hypertrophy. Arteriosclerotic heart disease
		57.3	1735†	+12.4				
8	72	53.2	1848*		Diagnosed during hospitalization	Good control	Gangrene of toes. Supracondylar amputation 2 days after Ke-2	Arteriosclerotic heart disease
		53.2	1750†	− 5.3				
		51.8	1894‡	+ 2.5				
					Serial determinations without KCl supplement			
9	49	66.8	1635		16 yrs.	In acidosis and coma with Ke-1; conscious after 2 days	Renal disease. Cerebral vascular accident	
		66.8	2168	+32.6				
		66.8	2133	+30.5				

TABLE I—*Continued*

Patient	Age	Weight	Ke	Change in Ke	Duration of diabetes	State of disease	Complications	Incidental diseases
		Kg.	*(mEq.)*	%				
					Single determinations			
10	23	39.5	1360		7 yrs.	Erratic control		Cirrhosis of liver by aspiration biopsy. Liver function tests normal
11	35	57.3	954		5 yrs.	Weight loss of 70 lbs. in 5 yrs. Regulation inadequate	Peripheral vascular disease and neuropathy	
12	50	86.8	3383		15 yrs.	Previously well controlled		Acute pneumonitis
13	58	45.8	2088		3 yrs.	Poor control	Neuropathy, obliterative vascular disease	
14	48	107.7	3148		14 yrs.	Fair control		Obesity
15	78	77.3	2092		Diagnosed during hospitalization			Recurrent urinary tract infection with prostatitis. Arteriosclerotic heart disease
16	59	87.0	2498		Diagnosed during hospitalization			Obesity, arteriosclerotic heart disease
17	67	74.3	2619		5 yrs.	Well controlled. Weight loss of 20 lbs. in 1 yr.	Obliterative vascular disease	Osteomyelitis
18	58	76.8	2160		3 yrs.	Poor control. 20 lbs. wt. loss in 10 mos.		
19	45	77.3	4125		15 yrs.	35 lbs. wt. loss in 2 yrs.		
20	65	77.3	2412		7 mos.		Neuropathy and generalized arteriosclerosis	

All Ke determinations were performed at weekly intervals unless otherwise noted.
* Ke-1, initial determination.
† Ke-2, determination after KCl, 3 gm. daily, for 6 days.
‡ Ke-3, determination 1 wk. after Ke-2.
§ Determination made 1 wk. after Ke-3.

The clinical course of the subjects is described briefly in Tables I and II.

In order to determine whether the average Ke/wt. in an unselected group of hospitalized diabetic subjects differed from that in normal subjects (Figures 1 and 2), the mean initial Ke/wt. value was compared with the mean value in 50 normal subjects. The mean value for Ke/wt. was significantly lower in the male diabetics than in normal males. The difference between the mean Ke/wt. values for diabetic and normal women (6) was not significant. A possible cause of this sex difference is discussed below.

The results of the serial determinations of Ke were as follows:

In 5 of the 15 individuals who were given an oral supplement of potassium, the value for Ke increased by more than 20 per cent. Two of the 5 subjects not given an oral supplement showed an increase in Ke of more than 20 per cent. An attempt was made to correlate the clinical status of these patients with the response of Ke to the administration of oral potassium, or to the routine therapy. Of the 7 subjects, 6 gave a history of poor regulation of the diabetes prior to admission and the seventh had a history of poor dietary in-

TABLE II

Exchangeable potassium content in diabetic females

Patient	Age	Weight	Ke	Change in Ke	Duration of diabetes	State of disease	Complications	Incidental diseases
		Kg.	*(mEq.)*	%				
					Serial determinations with KCl supplement			
21	54	50.4	1284*		7 mos.	Poor control		
		50.4	1945†	+51.5				
22	52	70.9	918*		13 yrs.	Fair control	Neuropathy and obliterative vascular disease	Acute pyelonephritis
		68.2	2217‡	+141.5				
		68.2	1954§	+112.9				
23	34	51.4	1773*		10 yrs.	35 lbs. wt. loss in 2 yrs. Erratic control		Multiple sclerosis, 8 yrs.
		52.3	2320†	+30.9				
24	45	72.3	2456*		7 yrs.	Erratic control. Well regulated for 2 wks. before Ke-1		Acromegaly
		72.3	2767†	+12.7				
25	62	71.4	1872*		5 yrs.		Neuropathy, retinopathy, hepatomegaly. Ulcers of toes	
		69.3	2062†	+10.1				
		70.5	1836‡	− 1.9				
26	27	54.8	1276*		4 yrs.	Very poor control. No recent wt. loss		
		54.8	1313†	+2.9				
27	63	43.9	1368*		5 yrs.	Poor control prior to hospitalization. 8 lbs. wt. loss in 6 mos.		
		44.1	1432†	+4.7				
		44.1	1591‡	+16.3				
					Serial determinations without KCl supplement			
28	55	68.6	927		9 yrs.	Fair control. Poor dietary intake	Ulcers of toes.	Arteriosclerotic heart disease
		68.2	2267‖	+144.6				
29	67	56.9	1469		6 yrs.	Fair control		Coronary insufficiency
		53.2	1356¶	−7.7				
		53.2	1333	−9.3				
30	59	72.7	1553		2 yrs.	Diabetic glucose tolerance curve		Postoperative myxedema. Anemia, 2 yrs.
		71.4	1576	+1.5				
31	56	70.0	1904		8 yrs.	Good control with diet alone		Hospitalized for headache and diplopia. Cerebral aneurysm
		70.1	1912	+0.4				

take. In most of these individuals, the increase in Ke could be ascribed to better regulation of the diabetes, or to replenishment of the body store of potassium by oral supplements or an adequate diet. There appeared to be no correlation between insulin dosage and the changes in Ke during the period of observation.

In 13 diabetics, the value for Ke did not change by more than 20 per cent. In 9 of these individuals, the diabetes was under good control; two required no insulin. Six patients were admitted for vascular complications resulting from diabetes—coronary insufficiency or peripheral vascular disease, with gangrene of the lower extremities; one had acromegaly, and four were hospitalized for unrelated disease processes. In 3 patients, mild diabetes was diagnosed for the first time during hospitalization.

COMMENT

The results of the present study suggest that the presence or absence of a potassium deficit in diabetes can be correlated roughly with the relative adequacy of control of the diabetes. It is

TABLE II—*Continued*

Patient	Age	Weight	Ke	Change in Ke	Duration of diabetes	State of disease	Complications	Incidental diseases
		Kg.	*(mEq.)*	%				
					Single determinations			
32	—	45.6	679		7 yrs.	Poor control, marked wt. loss	Neuropathy, renal disease, furunculosis	
33	55	55.9	2540		4 yrs.	Good control	Neurogenic bladder	
34	25	43.6	2201		9 yrs.	Erratic control		Psychosis
35	18	72.0	1673		3 yrs.	Fair control		Obesity, amenorrhea
36	59	77.6	1530		Diagnosed during hospitalization			Obesity, hypertension
37	59	53.6	1092		7 yrs.	No recent wt. loss		Admitted for coronary artery disease and angina
38	76	52.6	1662		3 yrs.	25 lbs. wt. loss in 5 yrs.		Polycythemia vera
39	70	39.5	1139		5 mos.	Poor control		
40	67	45.9	1976		Diagnosed during hospitalization	50 lbs. wt. loss in 15 mos.		Biliary cirrhosis
41	56	73.4	1750		7 yrs.	Poor control		Hepatosplenomegaly, cirrhosis, urinary tract infection, chronic cholecystitis
42	61	52.9	1317		17 yrs.	Fair control	Retinopathy, neuropathy, generalized arteriosclerosis	Admitted for gastrointestinal symptoms and signs

All Ke determinations were performed at weekly intervals unless otherwise noted.
* Ke-1, initial determination.
† K-2, determination after KCl, 3 Gm. daily, for 6 days.
‡ Ke-3, determination 1 wk. after Ke-2.
§ Determination made 3 wks. after Ke-3.
‖ One month interval between 2 determinations.
¶ Interval of 7 wks. between first 2 determinations.

recognized that the comparison of Ke values found in diabetics of various ages and normals of various ages, irrespective of difference in bodily habitus may not be valid. Many of the diabetics, in addition, had incidental diseases which in themselves may have influenced the Ke values.

No significant difference was found in the Ke/wt. value when the mean in the diabetic females was compared with that in normal females (6). It should be noted, however, that the mean in the normal from our laboratory is 9.2 mEq./kg. lower than that reported by Edelman (7). The range for this value reported by the latter, however, was greater than that in our series, suggesting that our data represent those from a more homogeneous group.

It has been previously shown that a potassium deficiency develops in the presence of a gross upset of carbohydrate metabolism. Atchley, Loeb and Richards (1) showed that, upon acute insulin withdrawal from known diabetics, the loss of large amounts of potassium in the urine was associated with the hyperglycemia and glycosuria. Although the authors attributed this effect to dehydration (since potassium loss is known to occur in other conditions producing dehydration) it is possible that some of this loss may be due to glycogenolysis.

Tissue breakdown results in the loss of potassium. An insulin deficiency, by decreasing the rate of glucose catabolism, would reduce the total amount of energy available to the organism and might thus produce some tissue breakdown, with the consequent loss of nitrogen and potassium. However, recent studies by Danowski and his coworkers (2), who compared the loss of potassium

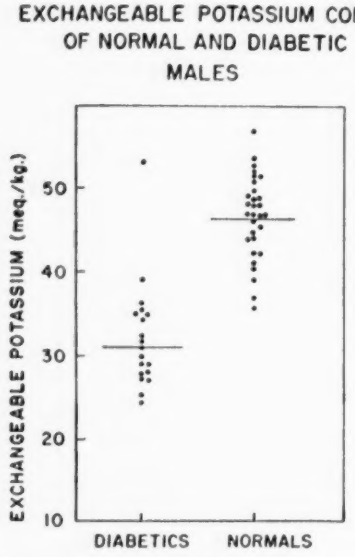

FIG. 1. THE MEAN KE/WT. IN NORMAL MALES IS 46.3 ± 4.31 MEQ./KG.; THAT IN THE DIABETIC MALES WAS 31.4 ± 7.25 MEQ./KG. $d = 14.9$, $s_d = 1.79$, $t = 8.32$, $P = < 0.01$

and nitrogen in acidotic diabetic patients, have shown that the potassium loss is too great to be attributed solely to the breakdown of tissue. It therefore appears that, in certain diabetic states, an intracellular deficiency of potassium develops.

Certain biochemical studies suggest that such a deficiency of potassium might be expected in poorly regulated diabetes. The maintenance of the differential concentration of sodium and potassium between the extra- and intracellular phases against a diffusion gradient requires the expenditure of energy. It has been shown that, in the red blood cell, this energy can be derived from glucose catabolism (8). There is an intimate relationship between the metabolism of potassium and that of carbohydrate. Although many of the factors involved in carbohydrate metabolism are not well understood, the following facts are known: The deposition of glycogen increases the potassium content of liver cells (9), whereas glycogenolysis results in a loss of potassium from hepatic tissue; the hepatic deposition of glycogen produces a decrease in the concentration of potassium and phosphate in the plasma; glycogenolysis has an opposite effect. Lack of insulin decreases hepatic glycogen deposition, presumably through an effect on hexokinase.

In-vitro studies of carbohydrate metabolism have yielded evidences suggesting that potassium is necessary for several specific enzymatic reactions. The amount of potassium in the cell may regulate the rate of these reactions; or, conversely, the rate of the reactions may determine the amount of potassium in the cell. A deficit of potassium may impair the resynthesis of carbohydrates (10). If the deficiency is far advanced, tissue glycogenesis does not occur.

In animals placed on diets deficient in potassium, the potassium content of the muscles decreases and the sodium content increases. The adrenal glands of such animals show an increase in weight. This finding suggests that the adrenal hormones affect the sodium and potassium content of cells. The specific role of the adrenal gland in the regulation of sodium and potassium metabolism in diabetics is not known at the present time.

The loss of potassium from the body in diabetic subjects, then, appears to be related to the following four processes: *1*) an excess of glycogenolysis over glycogenesis, *2*) the breakdown of

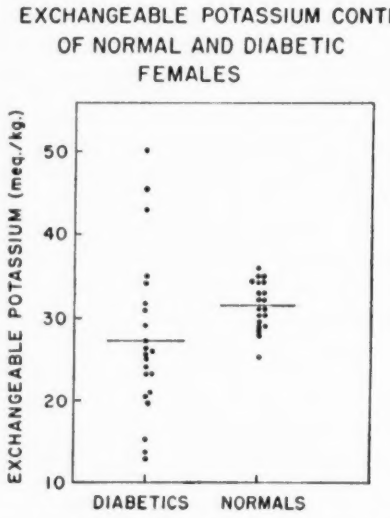

FIG. 2. THE MEAN KE/WT. IN NORMAL FEMALES IS 31.5 ± 2.90 MEQ./KG. (6); THAT IN THE DIABETIC FEMALES WAS 27.3 ± 9.77 MEQ./KG. THE MEAN DIFFERENCE, 4.2 MEQ./KG., WAS NOT STATISTICALLY SIGNIFICANT

tissue, *3*) dehydration, and *4*) disturbances in adrenal function.

The finding of a low Ke in a diabetic subject does not, however, necessarily indicate a metabolic deficiency of body potassium. Other possible explanations are: *1*) decrease in body mass, *2*) an increase in the relative fat content of the body, and *3*) expansion of the extracellular fluid compartment (4). Retention of potassium, however, would not be expected to occur except with correction of an intracellular metabolic deficiency of potassium or with impaired renal function.

The results of the present study suggest that whenever carbohydrate metabolism is not properly controlled in diabetes mellitus, an occult intracellular deficit of potassium may develop. It is not possible to determine from the data on hand whether this deficiency is due to superimposed dehydration and acidosis or whether it is due to the uncontrolled diabetes *per se*. Such a deficit may be corrected by better regulation of the diabetes (which permits adequate intake of potassium in the food) and the administration of an oral supplement of potassium.

SUMMARY AND CONCLUSIONS

The exchangeable potassium content (Ke) was determined by the radioactive isotopic technique in 42 unselected diabetic subjects. The initial Ke values were significantly lower in diabetic males than in normal male subjects. No significant difference was found in Ke between diabetic and normal female subjects.

Seven of the twenty subjects in whom serial determinations were performed showed an increase in Ke of more than 20 per cent. In general, the control of the diabetes was poorer in these seven subjects than in the 13 remaining individuals who did not show an increase in Ke. The results suggest that the presence or absence of a potassium deficit in diabetes can be correlated roughly with the relative adequacy of control of the disease.

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DISTRIBUTION AND FATE OF INTRAVENOUSLY ADMINISTERED MODIFIED HUMAN GLOBIN AND ITS EFFECT ON BLOOD VOLUME. STUDIES UTILIZING I^{131} TAGGED GLOBIN [1]

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The need for blood substitutes adequate to combat the fall in circulating volume in shock continues to stimulate search for high molecular weight compounds which might serve as plasma expanders. Globin, derived from human red blood cells, has been suggested for this purpose (1, 2). While considerable study with this substance is being pursued in various laboratories (1–7), there have been few published reports dealing with its use as a blood volume expander (1).

METHODS

The present investigation was approached along two lines:

1) Study of the fate and distribution of globin. Plasma and urine were analyzed at frequent intervals following the intravenous administration of tracer doses of I^{131} tagged globin accompanied by varying amounts of uniodinated globin. In vivo assay over the liver following the administration of the tagged globin was also performed in an attempt to localize the organ site of accumulation and breakdown. The disappearance rates of I^{131} tagged globin and albumin from intramuscular, subcutaneous, and intracutaneous sites of injection were determined for the purpose of comparing vascular permeability to the two proteins.

2) Blood volume studies with P^{32} tagged erythrocytes were performed during and after the intravenous administration of infusions of 32 and 48 grams of globin.

Radioiodinated proteins

Modified human globin [2] was iodinated with a mixture of I^{131} and I^{127} at pH 8.0 according to the method of Pressman and Eisen (9) and then dialyzed against frequently changed solutions of isotonic saline solution for approximately 72 hours. Assuming uniform distribution, the tagged protein contained 1 to 2 atoms of iodine per molecule of globin, based on its molecular weight of 34,000. At the time of use the activity ranged from 0.5 to 10.0 μc I^{131} per mgm. nitrogen. Iodinated globin was passed through Seitz filters and sterility was confirmed by culture before use. The administration of 25 mgm. of tagged protein to 2 rabbits failed to elicit evidence of toxicity within 72 hours.

Studies with various proteins have shown that at the level of iodination obtained, significant changes in the immunological properties of the protein do not occur (10). No significant differences between the dialyzed iodinated globin and the uniodinated globin used in this study were observed in the ultracentrifuge [3] and the electrophoretic pattern of a 1:1 mixture of globin and globin-iodine showed a single peak identical to that of globin alone. Ninety to ninety-seven per cent of the radioactivity was present in the precipitate after treatment with trichloracetic acid. After 3 hours of incubation of globin I^{131} with whole blood at 38° C. there was no increase in the nonprecipitable activity nor was there any detectable radioactivity in the centrifuged erythrocyte column above that attributable to trapped plasma.

Albumin I^{131} was prepared in the same manner or was obtained from Abbott Laboratories and dialyzed until the protein-free activity was less than 1 per cent of the total activity. Electrophoretic and ultracentrifugal analyses revealed no significant differences between this material and native albumin.

Subjects of these studies were convalescent and ambulant patients of the Veterans Administration Hospital, Bronx. None were acutely ill or in shock. All subjects received 5 drops of Lugol's solution 3 times per day during the studies. Assay over the neck showed this to be effective in inhibiting thyroid uptake of I^{131}.

Assay of radioactivity in plasma and urine

Approximately 5 ml. of isotonic saline solution containing 50 to 100 μc I^{131} and 10 to 30 mgm. protein was injected intravenously in the tracer studies. In vivo assay over the site of injection ruled out any invisible extravascular leak. Methods used in the assay of radioactivity

[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the results of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[2] Modified human globin was generously supplied by Sharp and Dohme, Incorporated, Glenolden, Pennsylvania. The preparation and characteristics of this protein have been described (8).

[3] Thanks are due to Dr. Kurt G. Stern, Brooklyn Polytechnic Institute for the ultracentrifuge analyses.

of the administered doses, whole blood specimens, plasma and erythrocyte fractions and urine were identical with those previously described for KI^{131} (11) and albumin I^{131} (12). The plasma and erythrocytes were separated after centrifugation for 10 minutes at 1000 g. Erythrocyte assays were done for all the points of curve I. B. and all but the last point of curve H. W. in Figure 1. The plasma activities at these times were corrected by subtracting from them 1.5 times the erythrocyte concentrations [4] after deduction of 5 per cent trapped plasma (13). I^{131} could be assayed in the presence of P^{32} without correction by using a bismuth walled gamma counter and thick glass Marinelli beakers. The total activity, trichloracetic acid-precipitable activity and the activity in the filtrate were determined in all urine specimens. Carrier globin or albumin was added to these specimens to facilitate precipitation of the minute amounts of protein bound I^{131} present.

Determination of globin concentrations

Globin nitrogen in serum was determined by the method of Kendall and Abell as described by Sample, Strumia, and Satta (15). The factor $7.5 \times N$ was used in calculating the globin concentrations. Recovery experiments revealed that 80 ± 3 per cent of the added globin could be accounted for in serum. The globin concentrations therefore were corrected by multiplying the observed values by 1.25. Only an occasional cloudiness without visual precipitate was noted on adding the reagents to globin-free serum.

Assay of relative change in radioactivity of liver

In vivo counting over the liver was performed with a bismuth walled Geiger counter enclosed in a ¾" lead housing containing an open window measuring 5" × 1" and placed 4¾" above the right upper quadrant of the abdomen of recumbent subjects. The position of the counter was kept unchanged during each period and a tapered lead shield which extended from the housing craniad to the

[4] This factor was based on the assumption that the erythrocyte penetrable fraction was distributed uniformly with regard to its concentration in plasma water and erythrocyte water as is the case with iodide (14). If the protein-free fraction were some molecule such as mono or diiodotyrosine which entered the erythrocytes in lower concentration, then this correction is actually not sufficient.

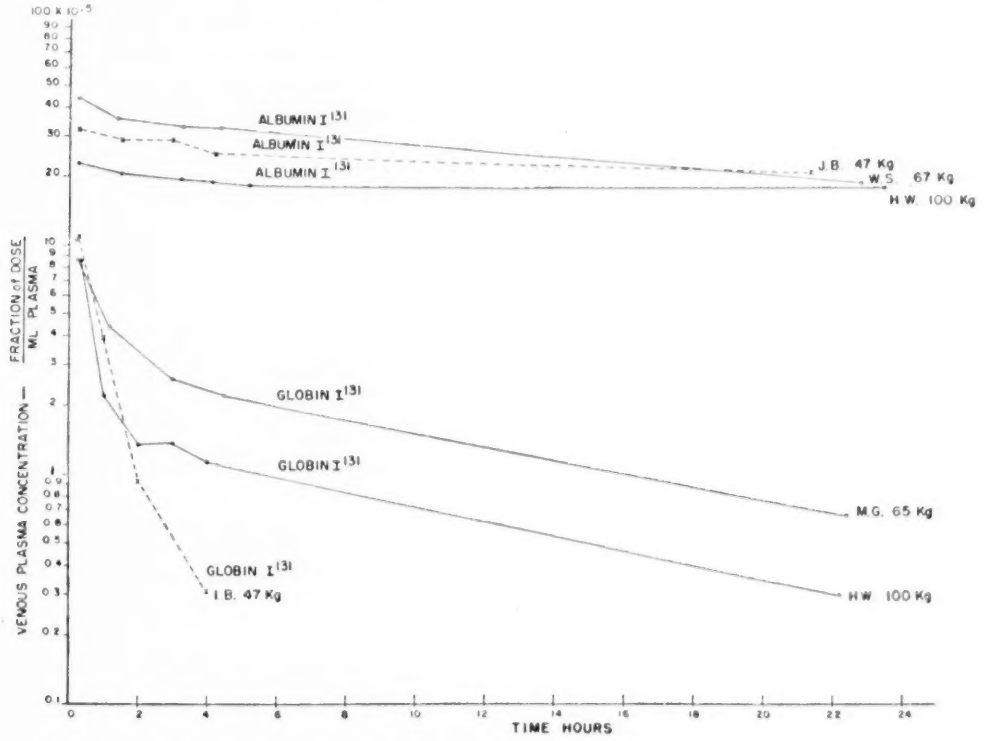

FIG. 1. VENOUS PLASMA CONCENTRATIONS EXPRESSED AS FRACTION OF THE DOSE ADMINISTERED PER ML. OF PLASMA FOLLOWING THE INTRAVENOUS ADMINISTRATION OF TRACER DOSES OF I^{131} TAGGED HUMAN SERUM ALBUMIN AND MODIFIED HUMAN GLOBIN

tube remained in contact with the skin. The scaler circuit leading from the counter fed into a Streeter-Amet printing register which automatically recorded the cumulative counts at one minute intervals.

Tissue clearance

The rate of disappearance of globin I^{131} and albumin I^{131} from intracutaneous, subcutaneous and intramuscular sites of injection in the lower extremities was studied by techniques similar to those previously described for Na^{24} (16). One-tenth ml. of solution containing 1–2 μc I^{131} and 100 to 1000 gamma of protein was injected in these studies.

Blood volume determinations

Initial total blood volumes, plasma volumes and red cell volumes were estimated from the dilution of P^{32} tagged erythrocytes (17, 18). The P^{32} activity was assayed using a thin glass walled beta gamma counter. Corrections were made for I^{131} activity, when present, by simultaneous assay of blood specimens in the gamma counter. The maximum correction necessary in case H. W. was less than 10 per cent and in case I. B. less than 1½ per cent of the P^{32} counting rates. A straight line describing the exponential biological decay curve of the tagged cells was established during a suitable control period. The changes produced by the infusion of globin and albumin were evaluated from the deviation of subsequent points from this line according to methods previously described (18). Changes in plasma volume were also independently calculated from the hematocrit and hemaglobin values according to the formula:

$$\frac{PV_n}{PV_0} = \frac{hgb_0 \times (1 - hct_n)}{hgb_n \times (1 - hct_0)}$$

where PV_0 is initial plasma volume and PV_n is any subsequent plasma volume (19). Hematocrits were performed in Wintrobe tubes spun for 30 minutes at 2000 g. Hemoglobin determinations were performed according to the method of Wong (20).

RESULTS

The rates of disappearance from plasma of intravenously injected tracer doses of globin I^{131} were very rapid when compared with those of albumin I^{131} (Figure 1). Thus at 12 minutes approximately two thirds of the globin activity had already disappeared from the intravascular circulation. By the following day only insignificant concentrations of circulating activity were observed.

At one hour (but not at 12–20 minutes) following injection of tracer doses of globin I^{131}, the red blood cells already contained activity well in excess of that which could be accounted for on the basis of trapped plasma. It should be appreciated that if the plasma correction factor, derived from the erythrocyte assays, were too small, the globin I^{131} curves would be even lower and steeper than shown since the ratios of erythrocyte activity/plasma activity increased with time. In the studies with albumin I^{131} significant activity in the erythrocytes was not encountered at any time.

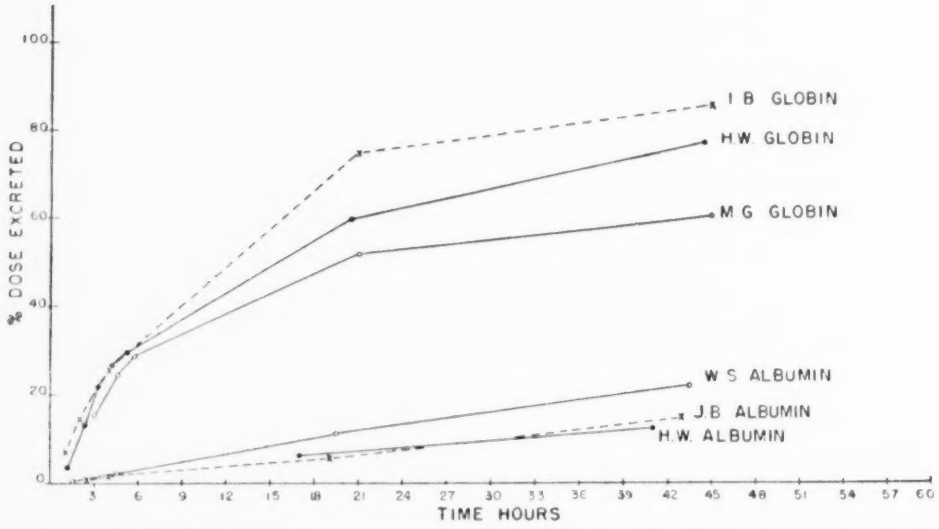

FIG. 2. CUMULATIVE URINARY EXCRETIONS OF I^{131} FOLLOWING THE INTRAVENOUS ADMINISTRATION OF TRACER DOSES OF I^{131} TAGGED HUMAN SERUM ALBUMIN AND MODIFIED HUMAN GLOBIN

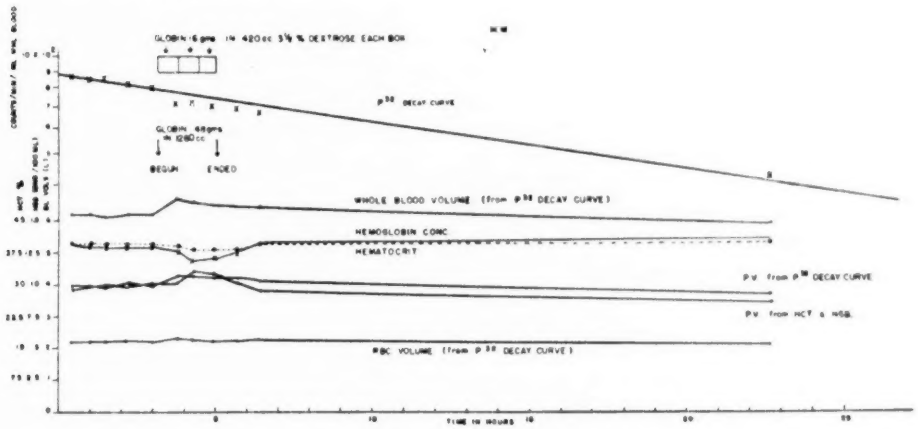
GLOBIN: 6 gms IN 420 cc 5% DEXTROSE EACH BOTTLE
P32 DECAY CURVE
GLOBIN: 48 gms IN 1260 cc
BEGUN
ENDED
WHOLE BLOOD VOLUME (From P32 DECAY CURVE)
HEMOGLOBIN CONC.
HEMATOCRIT
P.V. from P32 DECAY CURVE
P.V. from HCT & HGB.
RBC VOLUME (From P32 DECAY CURVE)
TIME IN HOURS

FIG. 3. BIOLOGICAL DECAY CURVE OF P^{32} LABELED ERYTHROCYTES, HEMOGLOBIN CONCENTRATION, HEMATOCRIT AND CALCULATED BLOOD VOLUMES BEFORE, DURING AND AFTER INTRAVENOUS ADMINISTRATION OF 48 G. OF GLOBIN IN SUBJECT H. W.

The cumulative urinary excretions of I^{131} expressed as per cent of the administered doses in these experiments amounted to approximately 32 per cent in the first 6 hours and 50–80 per cent in the first 21 hours (Figure 2) even though the collections in 2 of the patients (H. W. and M. G.) were known to be incomplete. Following the albumin I^{131} injections, only protein-free activity in the urine was detectable and did not exceed 10 per cent of the dose over the first 24 hours. Following injection of the tracer doses of globin I^{131}, 3 per cent to 10 per cent of the I^{131} was precipitable by trichloracetic acid in the urine passed up to four hours after injection. The urine passed after four hours did not contain more than 2 per cent precipitable I^{131}. Since the half time for nor-

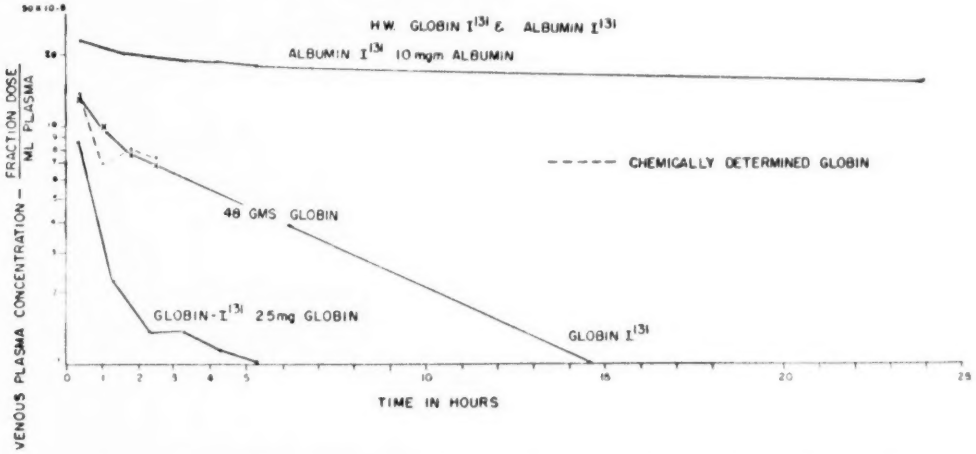
H.W. GLOBIN I131 & ALBUMIN I131
ALBUMIN I131 10 mgm ALBUMIN
CHEMICALLY DETERMINED GLOBIN
48 GMS GLOBIN
GLOBIN-I131 25mg GLOBIN
GLOBIN I131
VENOUS PLASMA CONCENTRATION — FRACTION DOSE / ML PLASMA
TIME IN HOURS

FIG. 4. VENOUS PLASMA CONCENTRATIONS OF UNTAGGED (INTERRUPTED LINE) AND TAGGED GLOBIN FOLLOWING THE INTRAVENOUS ADMINISTRATION OF GLOBULIN-I^{131} AT DIFFERENT CARRIER LEVELS AND OF TAGGED ALBUMIN AFTER A TRACER DOSE OF ALBUMIN-I^{131} IN SUBJECT H. W.

The 19 hour concentration of globin I^{131} (0.52×10^{-5}) was too low to be shown on the same scale. If extrapolated, the globin I^{131} line would pass through that point. At the same time serum globin was not detectable by chemical analysis.

mal renal removal of I^{131} as iodide is approximately 9 hours (11) when the thyroid uptake is blocked by iodine medication, the urinary excretion curves in the globin cases indicate that the I^{131} was very rapidly released from the protein. This confirms the impression obtained from the erythrocyte assays, that an appreciable fraction of the circulating activity was in non-protein bound form within an hour or two after injection.

The P^{32} decay curve and the changes in hematocrit, hemoglobin, and blood volume during and following the intravenous infusion of 48 g. of globin in 1260 cc. of 3½ per cent dextrose solution over a 2 hour period in subject H. W. are shown in Figure 3. A control study with 1000 cc of 5 per cent glucose in water performed in this patient showed no changes in the blood volumes. The maximum plasma volume increase during the globin infusion was 8 per cent falling to 5 per cent two hours after the infusion was completed. This is to be compared with a plasma volume increase of 35 per cent accompanying the intravenous administration of 50 g. of salt poor human serum albumin in 200 cc of buffered diluent in this same subject. The P^{32} decay curve from which the latter value was derived is published elsewhere (18).

The venous plasma concentration versus time relationship for globin I^{131} administered during the infusion of globin (after 16 g. had already been given) is shown in Figure 4 together with similar curves for the tracer doses of globin I^{131} and albumin I^{131} given without other protein. The concentrations of chemically determined globin during and after this infusion are also shown. The higher initial values and lower decline in concentration of globin I^{131} when administered together with a large amount of carrier globin as compared to the tracer doses alone are obvious. It should be noted, however, that the extrapolated globin I^{131} concentration at zero time even in the former case does not equal the early albumin con-

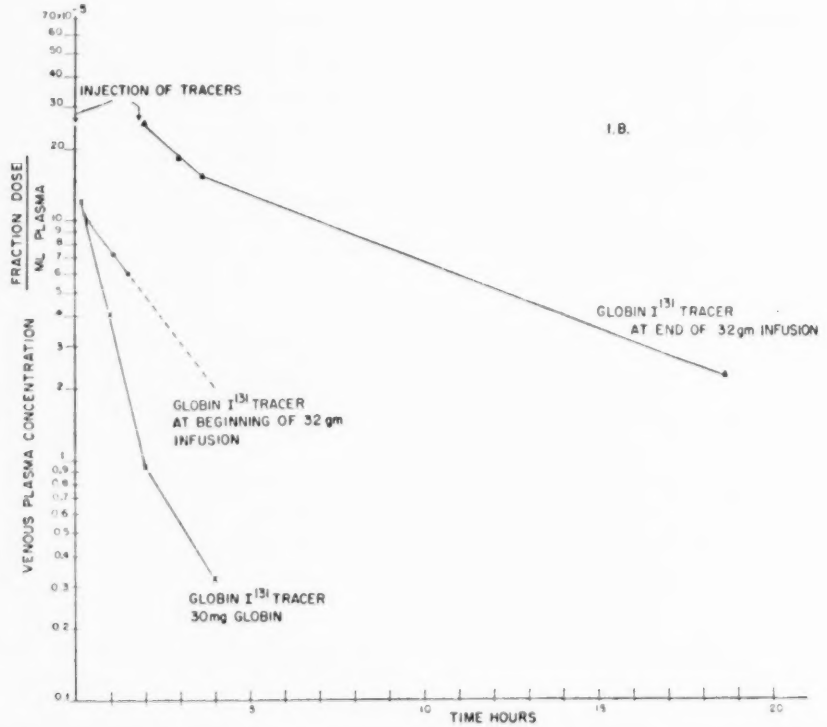
INJECTION OF TRACERS
I.B.
VENOUS PLASMA CONCENTRATION FRACTION DOSE / ML PLASMA
GLOBIN I[131] TRACER AT END OF 32gm INFUSION
GLOBIN I[131] TRACER AT BEGINNING OF 32 gm INFUSION
GLOBIN I[131] TRACER 30mg GLOBIN
TIME HOURS

FIG. 5. VENOUS PLASMA CONCENTRATIONS OF TAGGED GLOBIN FOLLOWING INTRAVENOUS INJECTION OF GLOBIN-I^{131} WITH DIFFERENT CARRIER LOADS OF GLOBIN IN SUBJECT I. B.

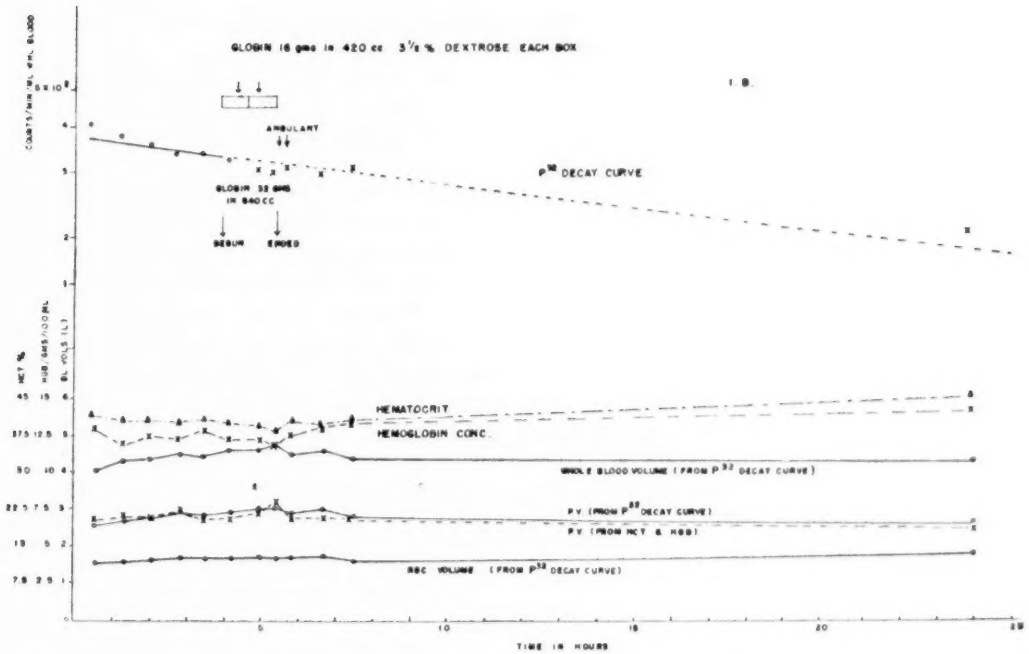

FIG. 6. BIOLOGICAL DECAY CURVE OF P^{32} LABELED ERYTHROCYTES, HEMOGLOBIN CONCENTRATION, HEMATOCRIT AND CALCULATED BLOOD VOLUMES BEFORE, DURING AND AFTER INTRAVENOUS ADMINISTRATION OF 32 G. GLOBIN IN SUBJECT I. B.

Since the rest period prior to the injection of the tagged cells was inadequate, there is an initial fall in P^{32} concentration, hematocrit and hemoglobin concentration due to temporary, continued hemodilution in the recumbent position. The reverse phenomenon (hemoconcentration) occurred during a brief period of unavoidable ambulation following discontinuance of the infusion.

centrations. This suggests that the globin is disappearing through some capillaries at a very rapid rate and that loss into these compartments is not prevented by the loading with large amounts of globin. Globin nitrogen could not even be detected in the plasma 18 hours after the termination of a 48 g. infusion. The close parallel between the tagged and untagged globin curves validates the usefulness of the former in these studies. It may also be noted that significant amounts of I^{131} were not present in the erythrocytes during the first 2½ hours.

Plasma levels of circulating globin following globin infusion

Subject	Time: From onset of infusion	Time: From end of infusion	Globin infused	Corrected globin conc.	Total circulating globin	Dose remaining in plasma
			g.	*mgm./ml. serum*	g.	%
H. W.	1 hr. 5 mins.		26	3.6	15.4	59.4
	1 hr. 45 mins.		44.8	3.1	13.2	29.4
	2 hrs. 30 mins.	40 min.	48	3.7	15.6	32.6
	3 hrs. 15 mins.	1 hr. 25 mins.	48	3.6	15.2	31.7
	20 hrs.	18 hrs. 10 mins.	48	0	0	0
I. B.	1 hr. 50 mins.	20 mins.	32	5.0	14.0	43.6
	2 hrs. 40 mins.	50 mins.	32	3.62	10.7	33.4
	3 hrs. 30 mins.	2 hrs.	32	4.66	12.8	39.8
	20 hrs.	18½ hrs.	32	0	0	0

Figures 5 and 6 illustrate the findings in similar experiments in subject I. B. Since this patient weighed 103 pounds, only 32 g. of globin in 840 ml. solution was administered. The tracer dose without other globin (bottom curve Figure 5) was administered 13 days before the other two doses. The middle curve describes the findings when the tracer globin I^{131} was administered immediately prior to the beginning of globin infusion. The concentration of activity following the globin I^{131} injected at the end of the infusion is shown in the upper curve. Since the I^{131} activity from the latter 2 doses of globin I^{131} could not be distinguished, the upper curve was obtained by subtracting the extrapolated activities of the middle curve from the total activities. Here again there is a marked difference among the rates of disappearance of globin I^{131} at different carrier levels. In this case also, globin was not detectable by chemical analysis on the morning following the infusion (18½ hours after injection). Table I summarizes the plasma globin data for H. W. and I. B.

In the experiments in which large amounts of carrier globin were administered together with tracer globin I^{131} an appreciable fraction of the early urinary losses of I^{131} was precipitable with trichloracetic acid (Figure 7), unlike the results obtained with the tracer doses alone. In the latter experiments the globin I^{131} was removed from the plasma with such rapidity that within an hour or two there was virtually no circulating globin I^{131} to filter through the glomeruli. However, when large doses of globin were administered concurrently with the tagged protein the plasma levels remained elevated for a longer period of time thus permitting larger amounts of undestroyed globin I^{131} to be excreted as such in the urine. The possibility that a renal threshold phenomenon could alone explain the findings is unlikely in view of the presence of some protein bound activity in the early urine specimens even

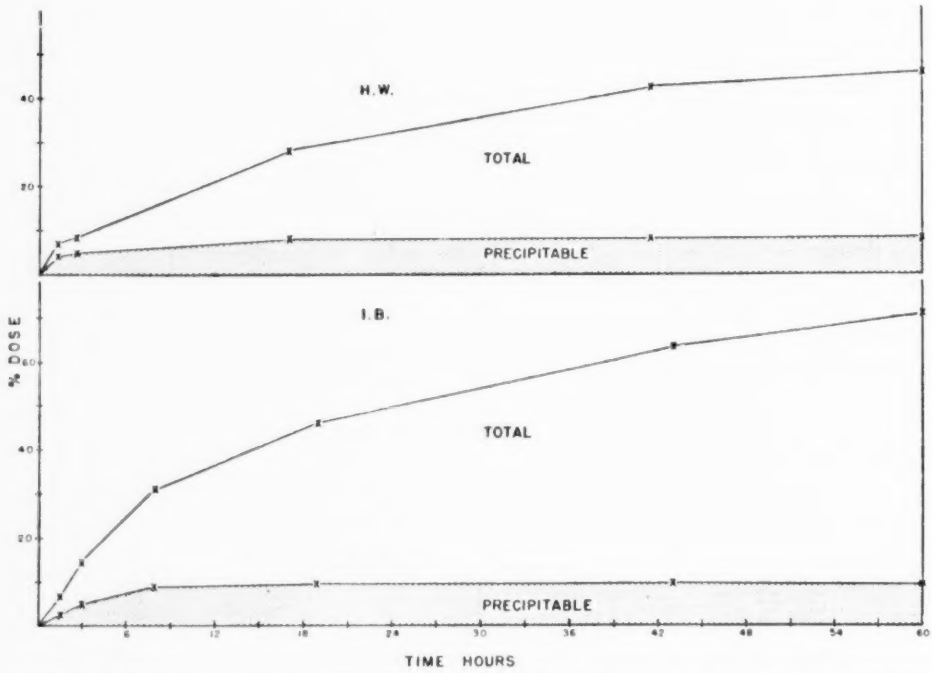

FIG. 7. CUMULATIVE URINARY EXCRETION OF PROTEIN-FREE AND PROTEIN-BOUND I^{131} FOLLOWING THE INTRAVENOUS ADMINISTRATION OF TRACER DOSES OF GLOBIN-I^{131} TOGETHER WITH LARGE LOADS OF UNTAGGED GLOBIN

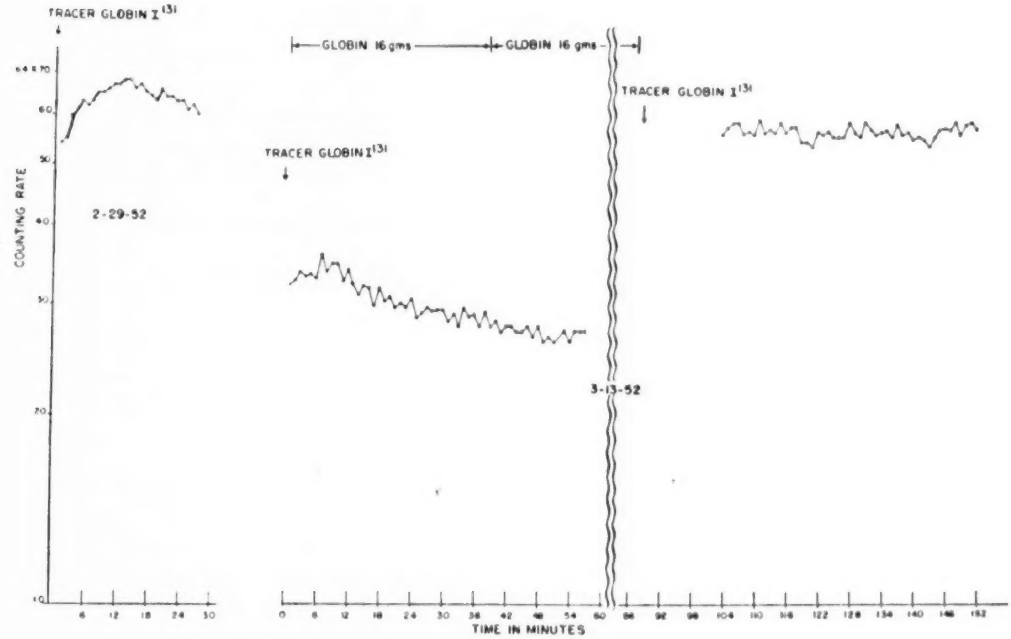

FIG. 8. COUNTING RATE OVER THE LIVER FOLLOWING THE INTRAVENOUS ADMINISTRATION OF GLOBIN-I^{131} WITH DIFFERENT UNTAGGED GLOBIN LOADS

The first segment shows the changes when tracer globin-I^{131} alone was administered. The counting rates following the administration of globin-I^{131} at the beginning and at the end of a 32 g. globin infusion are shown in the 2nd and 3rd segments respectively.

following the tracer doses which contained 30 mgm or less of protein.

Following administration of tracer globin I^{131} alone the radioactivity over the liver increased during the first 15 minutes and then gradually decreased (Figure 8). This pattern was confirmed by identical observations in another subject not included in this report and is suggestive of localization of globin in the liver followed by release of the non protein bound I^{131}. After the first ¼ hour the inflow and outgo become nearly equal and the succeeding slower decrease in activity over the liver may be attributed to gradual losses from the body. The more modest rise in activity when the globin infusion immediately followed the injection of the tracer globin I^{131} may be attributed to a slowing down of hepatic accumulation due to the additional load. The absence of any significant decline in activity for an hour following the third tracer dose which was given immediately at the end of a 32 gram globin infusion is a more emphatic demonstration of this "loading phenomenon." The differences in the plasma concentration curves following these 3 tracer doses may be taken as compatible with this view. Similar in vivo studies over the liver following albumin I^{131} injections showed no detectable change in counting rate over the first hour.

Figure 9 describes the disappearance of globin I^{131} and albumin I^{131} from muscle, skin and subcutaneous tissue. In most other globin I^{131} experiments and in some albumin I^{131} studies two component curves were observed. In all cases (11 globin and 17 albumin experiments), the rate of disappearance of globin I^{131} was of the order of 6 to 12 times that of albumin I^{131}. These experiments confirm the results of the plasma studies which sugested a more rapid loss of globin than albumin through capillaries in compartments other than the liver.

There were no untoward reactions following the globin administration.

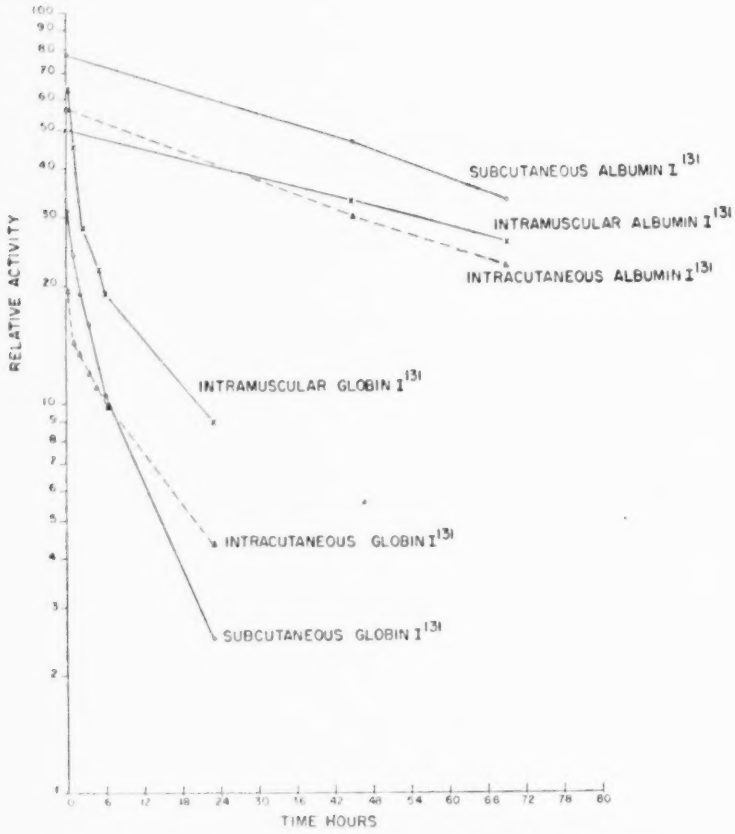

FIG. 9. CLEARANCE OF TAGGED GLOBIN AND ALBUMIN FROM TISSUES
The ordinate represents the relative counting rates over the site of injection.

DISCUSSION

A detailed consideration of the mechanism of globin I^{131} breakdown and of the release of the I^{131} is beyond the scope of this report. It may be suggested in brief, however, that release of mono-and diiodotyrosine occurs following proteolysis in the liver and that the subsequent metabolism of the amino acids with conversion of the I^{131} to iodide takes place with great rapidity as demonstrated by Albert and Keating (21).

The studies presented here suggest that globin is rapidly removed from plasma by the liver and by passage through capillaries throughout the body. While the relatively rapid clearance of globin I^{131} from injection sites in the skin, subcutaneous tissue and muscle of the lower extremities might be attributed to lymphatic drainage the process is nevertheless very much slower with albumin I^{131}. These data suggest a marked difference in permeability of small vessels to the two proteins. The low initial globin I^{131} plasma concentrations (at 12 minutes), even following large doses of globin, offer further evidence on this point.

The retardation of the disappearance of globin I^{131} from plasma by large doses of globin and the differences observed in the in vivo hepatic counting rates at different dosage levels demonstrate the effect of loading on the ability of the liver to concentrate and break down the globin. However, even with relatively large doses of globin (32 to 48 g.) the processes of removal and destruction were completed within a matter of hours. Under these conditions early urine specimens contained

significant fractions of trichloracetic acid precipitable I^{131}. The absence of such protein bound fractions after a few hours is further evidence of rapid disappearance of globin from the plasma.

The changes in plasma volume produced by the globin infusions were slight and in no way comparable to the increases which are observed regularly following albumin administration. In the 2 cases studied these changes could not be considered great enough to justify the use of globin in these amounts as a "plasma expander." The extremely rapid breakdown of the globin may indicate its usefulness as a ready source of amino acid nitrogen, but, if so, its superiority over protein hydrolysates has yet to be demonstrated.

SUMMARY

1. Radioiodinated modified human globin accompanied by varying amounts of carrier uniodinated globin was administered intravenously to human subjects. The rate of disappearance from plasma was inversely related to the quantity of globin administered. However even at the highest loads (32 to 48 g. given in 2 hours) approximately one half to two thirds of the globin had left the plasma at the conclusion of the infusion.

2. When large amounts of globin were infused, a small percentage (approximately 10 per cent) was excreted as such in the urine, but the major portion was rapidly metabolized. Suggestive evidence for the liver as the site of metabolic degradation is presented.

3. From a comparative study of the rates of disappearance from sites of injection into skin, subcutaneous tissues and muscle it may be inferred that the capillary wall is much more permeable to globin than to albumin. Plasma studies confirm this.

4. The changes in circulating plasma volume produced by intravenous globin administration were slight and transient when compared to the effect of similar doses of human serum albumin. The studies reported here have failed to establish the clinical usefulness of globin as a plasma expander.

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THE CHANGES IN CEREBRAL VASCULAR RESISTANCE OF MAN IN EXPERIMENTAL ALKALOSIS AND ACIDOSIS[1]

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Available evidence suggests that cerebral vascular resistance in man is primarily under chemical control. Past studies indicate that neurogenic stimuli, hormonal influences and most drugs have little ability to dilate cerebral vessels significantly. The potent chemical regulators which dilate cerebral vessels have been shown to be: *1*) anoxia and *2*) either CO_2 retention or decrease in the pH of arterial blood (1, 2). Using the nitrous oxide technique, Kety found increased cerebral blood flow (CBF) in subjects breathing 10 per cent O_2. He also showed that the increased CBF of respiratory acidosis produced by breathing 5 per cent or 7 per cent CO_2 was associated with increased CO_2 content and a lowered pH of arterial blood and decreased CBF of respiratory alkalosis following hyperventilation with a lowered CO_2 content and elevated pH.

Because of a tendency to an increase in CBF in late stages of diabetic acidosis, it was suggested originally by Kety, Polis, Nadler, and Schmidt (3) that the pH level might be more important than the CO_2 content in regulation of cerebral vascular tone. This viewpoint has been stated by others (4, 5). Some doubt about this interpretation is suggested by earlier findings of Bronk and Gesell (6). In 1927, they found that intravenous injection of sodium carbonate and sodium bicarbonate increased blood flow in the femoral and the carotid arteries of dogs under anesthesia.

The present study attempts to separate the effect on cerebral vessels of changes in pH and CO_2 content. By infusing $NaHCO_3$ and NH_4Cl intravenously, metabolic alkalosis and acidosis, respectively, were produced in man. The changes which occurred in CBF and cerebral vascular resistance were noted and compared to the changes previously reported after respiratory acidosis and alkalosis.

METHOD

The subjects studied were patients on the Medical Service of Duke Hospital. They were convalescing from a variety of illnesses and cannot be considered as a group of normal people. Six subjects were patients at Butner State Hospital. These patients were ill with schizophrenia and presented no physical illnesses. Metabolic alkalosis was produced by intravenous infusion of 1000 ml. of 3 per cent $NaHCO_3$ or 1000 ml. of 1.2 per cent $NaHCO_3$ over a period of sixty minutes. Metabolic acidosis was established by slower infusion of approximately 350 cc. of 0.8 per cent NH_4Cl intravenously over a period of 60 to 90 minutes. NH_4Cl caused slight hyperpnea, and nausea occurred if the speed of the infusion was increased. Changes in pulse rate and blood pressure did not occur if the drip rate was kept below the nausea threshold.

The effect of changes in blood volume was determined by administering 1000 ml. of 0.85 per cent NaCl solution intravenously over a period of one hour. The effects of both volume change and hypertonicity on the cerebral circulation were observed after the intravenous administration of 1000 ml. of 2 per cent NaCl solution over a period of one hour.

Cerebral blood flow (CBF) was measured, using the nitrous oxide technique described by Kety and Schmidt (7) and modified by Scheinberg and Stead (8). Oxygen differences in volume per cent ($A\text{-}VO_2$) were determined by the method of Hickam and Frayser (9) on the integrated arterial and cerebral venous blood specimens, respectively, or on the arterial and venous blood samples obtained immediately before and after the patient breathed N_2O. Cerebral metabolic rate of oxygen consumption ($CMRO_2$) in cubic centimeters of oxygen used per minute, per 100-grams of brain, was calculated by multiplying the rate of CBF in cc./min./100 gms. of brain by the $A\text{-}VO_2$ differences in volumes per cent. Arterial blood pH changes were determined anaerobically by a glass electrode, using a Beckman pH meter, and the readings were corrected to 37° C. Arterial blood hemoglobin levels were measured, using spectrophotometric methods. Blood pressure readings were made by the auscultatory method, using a sphygmomanometer with the arm held at heart

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TABLE I

Effect of a 60-minute intravenous infusion of 1000 ml. of isotonic NaCl (0.9%) in six subjects

	Control	*S.D.	†S.E.	Experiment	S.D.	S.E.	p Value
CBF ml./min./100-grams brain	60	±17	±7	60	±14	±6	n.s.
$A-VO_2$ Difference vols. %	6.5	±1.3	±.5	6.2	±.9	±.4	n.s.
$CMRO_2$ ml. O_2 used/min./100-grams brain	3.7	±.5	±.2	3.7	±.6	±.2	n.s.
CVR (mean arterial BP/CBF)	1.7	±1	±.6	1.6	±.8	±.5	n.s.
Arterial pH units	7.36	±.01	±.005	7.35	±.01	±.005	n.s.
Arterial Hemoglobin grams %	14.0	±1.1	±.5	12.7	±1	±.5	.05

* Standard Deviation $= S = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{N}}{N-1}}$ † Standard Error $= \frac{S}{\sqrt{N}}$

level. The mean arterial blood pressure was calculated by adding one-third of the pulse pressure to the diastolic blood pressure.

In each study, the CBF was measured by the N_2O technique, both before and during infusions of $NaHCO_3$, NaCl, and NH_4Cl. In five instances after infusion of $NaHCO_3$, and in one instance after infusion of NH_4Cl, the patient breathed 5 per cent CO_2 for four minutes. In these six instances, the changing $A-VO_2$ difference before and during CO_2 inhalation was measured. From the change in $A-VO_2$ difference, a third cerebral blood flow was estimated utilizing the fact that during 5 per cent CO_2 inhalation, $CMRO_2$ remains constant (*2*).

RESULTS

Control studies with saline

The results recorded in Table I show that intravenous infusion of 1000 ml. of isotonic (0.9 per cent) saline over sixty minutes has no effect on CBF, or $CMRO_2$, $A-VO_2$ difference, or pH of arterial blood. Significant decreases in hemoglobin values occur, indicating a measurable degree of hemodilution. Table II shows the results in six patients after infusion of 1000 ml. of 2 per cent NaCl. This is approximately isotonic with a 3 per cent solution of $NaHCO_3$. There is a 10 per cent, but statistically insignificant, increase in CBF. A significant narrowing in $A-VO_2$ difference does occur. This, combined with the directional change in cerebral blood flow, suggests that hypertonic saline solution does increase cerebral blood flow. We have no ready explanation for this effect. Hemodilution of approximately the same magnitude occurred with both isotonic and hypertonic saline, and neither changed pH significantly.

Metabolic alkalosis

Tables III and IV show the effect of intravenous infusions of approximately 1000 ml. of isotonic (1.2 per cent) and hypertonic (3 per cent $NaHCO_3$), respectively. Similar results occur in both instances, but the changes are greater after 3 per cent $NaHCO_3$, suggesting a quantitative action of the bicarbonate ion. The increase in blood flow with hypertonic $NaHCO_3$ solution is greater than that produced by hypertonic NaCl solution. The sharp increase in CBF after 3 per cent $NaHCO_3$ is comparable to that found after inhalation of 5 per cent CO_2. The increased CBF after bicarbonate infusion is associated with an increased pH of arterial blood. The increased CBF after CO_2 inhalation is associated with decreased pH of arterial blood.

Although CO_2 tensions were not measured in the $NaHCO_3$ infusion experiments reported here, other studies (10–12) suggest that after $NaHCO_3$ infusion, the CO_2 tension changes are minimal, while changes in the bicarbonate ion content are great. The absence of visible changes in respiratory rate and depth after $NaHCO_3$ infusion also suggests little change in CO_2 tension.

TABLE II

Effect of a 60-minute intravenous infusion of 1000 ml. of hypertonic NaCl (2.0%) in seven subjects

	Control	S.D.	S.E.	Experiment	S.D.	S.E.	p Value
CBF ml./min./100-grams brain	59	±12	±5	66	±16	±6	n.s.
A−VO_2 Difference vols. %	6.2	±.95	±.4	5.0	±.5	±.2	.01
$CMRO_2$ ml. O_2 used/min./100-grams brain	3.6	±.5	±.2	3.3	±.8	±.3	n.s.
CVR (mean arterial BP/CBF)	1.5	±.4	±.1	1.4	±.4	±.2	n.s.
Arterial pH units	7.35	±.01	±.007	7.33	±.02	±.01	n.s.
Arterial Hemoglobin grams %	13.0	±1.4	±.6	11.5	±1	±.4	.05

TABLE III

Effects of a 60-minute intravenous infusion of 1000 ml. of isotonic $NaHCO_3$ (1.2%) in six subjects

	Control	S.D.	S.E.	Experiment	S.D.	S.E.	p Value
CBF ml./min./100-grams brain	60	±12	±5	78	±18	±7	.1 to .05
A−VO_2 Difference vols. %	6.1	±.8	±.3	5.1	±.7	±.3	.02
$CMRO_2$ ml. O_2 used/min./100-grams brain	3.6	±.6	±.2	3.9	±.5	±.2	n.s.
CVR (mean arterial BP/CBF)	1.6	±.3	±.05	1.2	±.2	±.04	.05
Arterial pH units	7.39	±.01	±.004	7.48	±.01	±.005	.01
Arterial Hemoglobin grams %	12.3	±.8	±.3	11.3	±1.7	±.7	n.s.

TABLE IV

Effect of a 60-minute intravenous infusion of 1000 ml. of hypertonic $NaHCO_3$ (3%) in ten subjects

	Control	S.D.	S.E.	Experiment	S.D.	S.E.	p Value
CBF ml./min./100-grams brain	52	±11	±3	88	±15	±5	.01
A−VO_2 Difference vols. %	6.9	±1.4	±.4	4.2	±.7	±.2	.01
$CMRO_2$ ml. O_2 used/min./100-grams brain	3.6	±1.1	±.3	3.4	±.8	±.3	n.s.
CVR (mean arterial BP/CBF)	1.8	±.4	±.1	1.1	±.3	±.1	.01
Arterial pH units	7.34	±.03	±.01	7.52	±.02	±.006	.01
Arterial Hemoglobin grams %	13.6	±1.3	±.4	12.2	±1.4	±.4	.02

TABLE V

Effect of a 60 to 90-minute intravenous infusion of 350 cc. of NH_4Cl (0.8%) in seven subjects

	Control	S.D.	S.E.	Experiment	S.D.	S.E.	p Value
CBF ml./min./100-grams brain	66	±12	±5	52	±8	±3	.05 to .02
A−VO_2 Difference vols. %	6.3	±.5	±.2	8.6	±.8	±.3	.01
$CMRO_2$ ml. O_2 used/min./100-grams brain	4.2	±.7	±.3	4.4	±.5	±.2	n.s.
CVR (mean arterial BP/CBF)	1.3	±.3	±.1	1.6	±.5	±.1	.01
Arterial							
pH / units	7.36	±.014	±.005	7.32	±.016	±.006	.05
CO_2 content / vols. %	50	±3	±1	46	±3	±1	.1 to .05
Hemoglobin / grams %	13.0	±2	±.7	12.4	±2	±.6	n.s.
p CO_2 / mm. Hg	48	±7	±3	47	±5	±2	n.s.

Metabolic acidosis

In Table V are recorded the effects of the slow infusion of 0.8 per cent NH_4Cl on the cerebral circulation. There is a reduction in blood flow, the A-VO_2 difference widens greatly, and no change in $CMRO_2$ occurs. There is a consistent drop in arterial pH and serum CO_2 content and very little change in CO_2 tension. Thus, metabolic acidosis, as produced by NH_4Cl is accompanied by a reduction of CBF, in contrast to respiratory acidosis in which CBF is increased. Metabolic acidosis produced in some other fashion than by HN_4Cl should be studied. It is possible that we are really measuring, in part, the effects of NH_4 ion rather than the uncomplicated effects of acidosis.

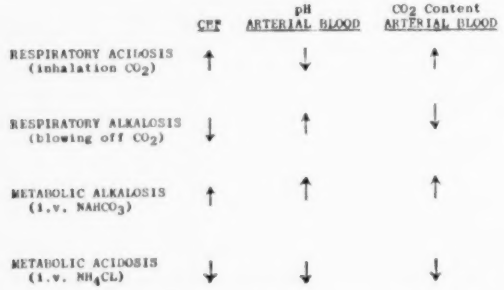

FIG. 1. THIS CHART SHOWS THE DIRECTIONAL CHANGES IN CBF ARTERIAL PH AND ARTERIAL CO_2 CONTENT AS FOUND BY KETY AND SCHMIDT IN RESPIRATORY ACIDOSIS AND ALKALOSIS AND BY THIS STUDY IN METABOLIC ALKALOSIS AND ACIDOSIS

Carbon dioxide inhalation in metabolic alkalosis and acidosis

In Figures 1 and 2 are schematically drawn the effect of CO_2 when breathed by alkalotic and acidotic subjects, respectively. When the subject who is alkalotic from $NaHCO_3$ breathes CO_2, a sharp further increase in CBF occurs while pH returns toward, but does not quite reach, pre-bicarbonate levels. Similarly demonstrated, but in a reverse fashion, are the results (Figure 2) in a metabolic acidotic subject who breathes CO_2. Here, CBF increases above pre-ammonium chloride levels while pH becomes even lower.

DISCUSSION

Sodium bicarbonate increases cerebral flow by decreasing cerebral vascular resistance. NH_4Cl decreases cerebral blood flow by increasing cerebral vascular resistance. Earlier studies (*2*) have shown that increased and decreased amounts of carbon dioxide in the arterial blood increase and decrease CBF, respectively. The summary chart

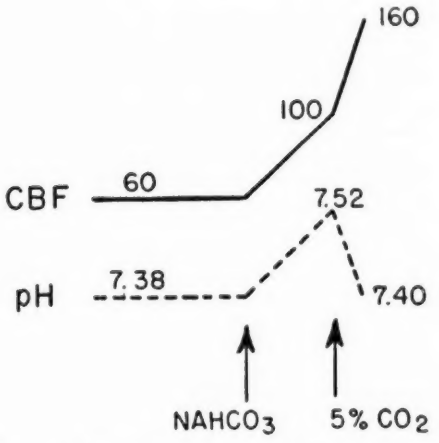

FIG. 2. SCHEMATICALLY DRAWN ARE THE CHANGES IN CBF AND pH OF ARTERIAL BLOOD BEFORE AND DURING THE ADMINISTRATION OF 100 6 ML. OF 3 PER CENT $NaHCO_3$ OVER A 60-MINUTE TIME PERIOD

At the second arrow, 5 per cent CO_2 was inhaled and the changes recorded occurred after four minutes.

(Figure 3) indicates by arrow the directional changes in CBF, pH and CO_2 content which occur in respiratory acidosis and alkalosis as described by Kety, and metabolic alkalosis and acidosis as found in this study. When one compares the directional changes in arterial blood pH with cerebral blood flow changes, no correlation exists. However, when one compares the directional changes in total CO_2 content of arterial blood with those of CBF, one finds complete correlation.

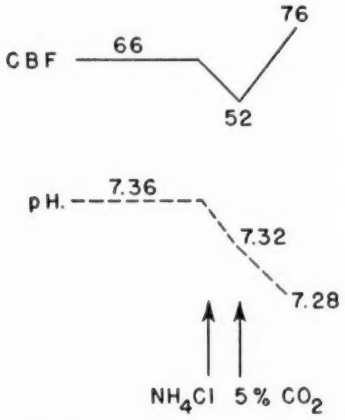

FIG. 3. SCHEMATICALLY DRAWN ARE THE CHANGES IN CBF AND pH OF ARTERIAL BLOOD BEFORE AND AFTER INTRAVENOUS INFUSION OF 0.8 PER CENT NH_4Cl OVER A PERIOD OF 60 TO 90 MINUTES

At the second arrow 5 per cent CO_2 was inhaled and the changes recorded occurred after four minutes.

CONCLUSIONS

1. Metabolic alkalosis, as produced by giving 3 per cent $NaHCO_3$ solution intravenously, increases CBF 65 per cent above resting normal values; isotonic bicarbonate solution (1.2 per cent) causes a 30 per cent increase.

2. The increase in CBF after $NaHCO_3$ is not primarily due to changes in intravascular volume because an equivalent degree of hemodilution produced by the infusion of isotonic NaCl solution does not cause an increase in CBF.

3. Infusion of 2 per cent NaCl solution does cause a demonstrable increase in CBF. It is not of the order of magnitude produced by the infusion of an equally hypertonic solution of $NaHCO_3$.

4. Intravenous infusion of 0.8 per cent NH_4Cl solution reduced CBF approximately 20 to 25 per cent.

5. These studies, when coupled with previous studies of Kety, show that—in the absence of anoxia—cerebral vascular tone is more closely related to total CO_2 content of arterial blood rather than arterial pH levels.

ACKNOWLEDGMENTS

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TYPE SPECIFIC MENINGOCOCCIC AGGLUTININS. III. APPLICATION OF THE TEST TO SPORADIC CASES AND TO CLINICALLY TYPICAL CASES LACKING BACTERIOLOGICAL PROOF [1]

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INTRODUCTION

The type specific meningococcic agglutinin content of human serums has been under investigation since March 1942 at the Bureau of Laboratories of the Department of Health of the City of New York. The first paper of this series was published in 1944 (1) in which the method of testing was described in detail and certain limitations of the test were discussed. The second report in 1945 (2) showed the relationship of the titers to the course of the disease. It was shown that meningococcic agglutinin production followed the classical curve of the other febrile diseases. Particular emphasis was placed on a study of bacteriologically proved cases. The observations were made at a time when the incidence of meningococcic infection due to Group 1 was comparatively high. It was, therefore, considered important to determine whether this test would have equal value if used on sporadic cases when the infecting organisms might be less antigenic and under all other conditions of a long range study. Continuation of the investigation was further stimulated by the fact that the cultural methods usually employed to detect the etiological agent were often unsuccessful following sulfonamide and antibiotic therapy.

METHOD

The method used in this study has been described in our previous papers (1, 2). Briefly, all serums were tested for Group 1, Group 2-alpha, and Group 2 agglutinins. The cultures used for the antigens were standard stock strain cultures of well established specificity and antigenicity. Serum dilutions ranged from 1/50 to 1/1,600. Each dilution was increased twofold. The tests were incubated at 37° C. for 2 hours, centrifuged at high speed for ten minutes and kept in the refrigerator overnight. Readings were made after centrifugation and also following overnight storage. The last tube showing definite (2 plus) clumping was considered indicative of the titer of the serum. Agglutinin titers over 1/100 were regarded as significant of the presence of specific antibody, whereas those up to and including 1/100 were considered within the normal range.

MATERIALS

Since March 1942, 4,272 serums collected from 2,826 individuals have been tested for type specific meningococcic agglutinin content.[2] The sources of these specimens were 407 cases of bacteriologically proved meningococcic infection, 1,269 cases of suspected meningococcic infection, 211 individuals suffering from gonococcic infection, 77 instances of exposure to active meningococcic infection, 387 cases of febrile diseases caused by organisms other than the meningococcus, and as controls 465 serums sent to the laboratory for routine syphilis serology.

RESULTS

The distribution of meningococcic agglutinins among the various groups of subjects studied during the ten year period followed essentially the same pattern observed in the initial three year investigation (Table I). It should be noted that results included in this table represent individuals tested one or more times. Significant titers were observed in 72.9 per cent of the 407 bacteriologically proved cases of meningococcic infection. The 1,269 cases of suspected meningococcic infection were placed in two groups. The first group consisted of 133 instances in which clinical symptoms were typical but bacteriological confirmation was lacking. Significant agglutinins were observed in 81.2 per cent of these cases. The second group of 1,136 cases included those with doubtful clinical diagnosis. Only 47 per cent of these cases showed agglutinins in significant titers. It is probable that a number of the infections in this group were caused by organisms other than the meningococcus. Twenty-three and three

[1] Presented at the Fifty-second General Meeting of the Society of American Bacteriologists, May 29, 1952.

[2] We are indebted to Dr. Erwin Neter of the Children's Hospital, Buffalo, N. Y., for supplying us with serums and the complete clinical resumés on more than 200 cases included in this study.

TABLE I

Results of meningococcus agglutinin content tests on individuals tested from 1942–1952, New York City

Source of serum	Number of cases tested	Serums showing agglutinins in significant titers	
		No. of cases	*per cent*
1. Bacteriologically proved cases of meningococcic infection	407	297*	72.9
2. Suspected meningococcic infection, but lacking cultural proof			
a. Clinically typical cases	133	108†	81.2
b. Clinically doubtful cases	1,136	534‡	47.0
3. Cases of gonococcic infection	211	47‖	23.3
4. Exposures to active meningococcic infection	77	12§	15.6
5. Cases of febrile disease caused by organisms other than the meningococcus	387	21¶	5.4
6. Unselected serums sent to the laboratory for routine syphilis serology	465	0	0

* 228 cases showed agglutinins for Group 1; 10 cases for Group 2; 13 cases for Group 2-a; 38 cases for Groups 1 and 2-a; and 8 cases for Groups 1, 2, and 2-a.

† 87 cases showed agglutinins for Group 1; 2 cases for Group 2; 7 cases for Group 2-a; 10 cases for Groups 1 and 2-a; and 2 cases for Groups 1, 2, and 2-a.

‡ 396 cases showed agglutinins for Group 1; 6 cases for Group 2; 54 cases for Group 2-a; 61 cases for Groups 1 and 2-a; 7 cases for Groups 1, 2 and 2-a; 7 cases for Groups 1 and 2; and 3 cases for Groups 2 and 2-a.

‖ 42 cases showed agglutinins for Group 1; 3 cases for Group 2-a; and 2 cases for Groups 1 and 2-a.

§ 7 instances showed agglutinins for Group 1; 4 for Group 2-a; and one for Groups 1 and 2-a.

¶ 19 cases showed agglutinins for Group 1; and 2 cases for Groups 1 and 2-a.

tenths per cent of 211 instances of gonococcic infection also showed agglutinins in significant titers, as well as 15.6 per cent of the 77 individuals exposed to active meningococcic infection. On the other hand, only 5.4 per cent of 387 cases of febrile disease caused by organisms other than the meningococcus showed meningococcic agglutinins. The positive findings were noticed in the following disease states: influenzal meningitis (5 cases), pneumococcic meningitis (4 cases), tuberculous meningitis (2 cases), and in luetic meningitis, sympathic meningitis with brain tumor, three different forms of encephalitis, poliomyelitis, Rocky Mountain Spotted Fever, measles, staphylococcic sepsis, and erythema nodosum (one case each). One can only speculate on the significance of these few positive results. It is possible that the apparent false-positives may be contacts to active meningococcic infection or harbor meningococci which indicate latent infection not the basic cause of the current illness. Agglutinins for Group 1 meningococci were the predominant type encountered in each group. Group 2-a agglutinins alone or in combination with Group 1 were observed in from 10 to 40 per cent of the cases in each group. Agglutinins for Group 2, however, were found only in 10 per cent of the bacteriologically proved or suspected cases of meningococcic infection. In contrast to these findings, no agglutinins in titers over 1 to 100 were observed in any of the 465 control serums sent to the laboratory for routine syphilis serology.

As the investigation progressed it became apparent that for optimum results successive specimens must be tested during the various phases of the disease. It will be noted that when such serial tests were made on three or more specimens in either the group of bacteriologically proved cases or in the group of clinically typical cases lacking such proof, the magnitude of positive results exceeded 95 per cent (Table II). In the same two groups positive findings were observed in 84 per cent of the cases when two specimens were tested, and could be expected only in approximately 60 per cent of the cases when a single serum was available. While there was a slight rise in the per cent of positive findings in the group of individuals suffering from febrile diseases caused by organisms other than the meningococcus, the number of instances in which three or more specimens were available was comparatively small and cannot be considered statistically significant. In one of these instances the case may be considered a contact since it was in a crib next to a meningococcic meningitis patient. Another subject yielded negative serums until the 15th day, followed by a gradual increase in titer from the 17th

TABLE II

Relationship of the appearance of significant titers to the number of successive specimens tested at intervals of from three to seven days

Clinical diagnosis	Number of specimens tested	Number of cases	Type specific meningococcic agglutinins observed in titers over 1/100	
			No. of cases	*per cent*
1. Bacteriologically proved meningococcic infection	1	212	120	56.6
	2	102	85	83.3
	3 or more	93	92	98.9
2. Suspected meningococcic infection				
a. Clinically typical	1	50	32	64.0
	2	37	32	86.0
	3 or more	46	44	95.7
b. Clinically doubtful	1	815	316	38.8
	2	222	145	64.4
	3 or more	99	75	75.7
3. Febrile diseases caused by organisms other than the meningococcus	1	283	13	4.5
	2	72	4	5.5
	3 or more	32	4	12.5

to the 19th day. In the third case, the first test was made 78 days after the onset of illness.

The practical application of the meningococcus agglutination test and the individual variation which occurred can best be illustrated by presenting protocols of a number of clinically typical cases without bacteriological proof (Table III).

Agglutinins corresponded in type with the infected organisms in every case where the organism was isolated and typed. This fact as well as the

TABLE III

Protocols of individual cases of clinically typical meningococcic infection lacking bacteriological proof

Case No.	Clinical summary	Laboratory findings	Days after onset	Agglutinin titer 1: Group 1	Group 2 a	Group 2
1	*History:* White ♀ aged 18 years, admitted Jan. 10, 1947, sudden onset Jan. 8, headache, vomiting, semi-stupor, delirium. Temp. 104° *Examination:* Rigid neck, Brudzinski +, Kernig + *Clinical impression:* Meningococcic meningitis *Treatment:* Sulfadiazine *Outcome:* Recovered	*Spinal fluid:* Protein 710 mg.% Sugar 0 mg.% Large no. polys. Smear—negative Culture—no growth	2 5 7	400 800 1,600	0 0 0	0 0 0
2	*History:* White ♂ aged 4 years, admitted Jan. 1, 1948, sudden onset Dec. 31, circulatory collapse that improved with stimulation. Temp. 105° *Examination:* Rigid neck, Brudzinski +, Kernig +, typical rash *Clinical impression:* Meningococcic meningitis *Treatment:* Sulfadiazine and penicillin *Outcome:* Recovered	*Spinal fluid:* Protein 300 mg.% Sugar 10 mg.% Large no. polys. Smear—negative Culture—no growth	2 5 9 12	0 0 0 0	0 0 0 0	0 100 800 1,600
3	*History:* Colored ♂ aged 3 weeks, admitted July 19, 1945, sudden onset July 18, irritable, convulsions, crying, rash. Temp. 103° *Examination:* Typical meningococcic rash *Clinical impression:* Meningococcemia *Treatment:* Sulfadiazine *Outcome:* Recovered	*Blood culture:* Negative	7 19, 26 36, 41 55 57 66	0 1,600 400 1,600 800 400	0 0 0 0 0 0	0 0 0 0 0 0

0—Titer less than 1/50

TABLE IV

Protocols on three brothers admitted to the hospital with symptomatology of acute meningococcemia

Case	Blood culture	Date of onset	Agglutination with Group 1 meningococci Days after onset on which serum was collected										
			1	2	8	10	15	20	34	43	45	70	83
M. M.	Group 1 meningococcus	Dec. 18, 1950	0	—	200	1,600	1,600	1,600*					
J. M.	No growth	Dec. 18, 1950	—	0	800	1,600	1,600	1,600*					
L. M.	No growth	Dec. 18, 1950	—	200	400	800	1,600	1,600†	1,600	400	800	100	0

0—Titer less than 1/50
— Not tested

* Discharged from hospital—recovered
† Showed indications of pulmonary tuberculosis

high percentage of positive findings observed in the group of clinically typical cases lacking bacteriological proof suggested that the test could be used in tracing the epidemiology of an outbreak. The effectiveness of this method was illustrated by the case of three brothers admitted to the hospital with symptoms of acute meningococcemia on December 10, 1950 (Table IV). A Group 1 meningococcus was recovered from only one of these patients. Serums were collected from each patient at regular intervals. By the eighth day one showed a titer of 1 to 200 for Group 1 meningococci, the second a titer of 1 to 400 and the third a titer of 1 to 800. All reached a maximum titer of 1 to 1,600 by the 15th day. Final serums were received from two of the brothers (M. M. and J. M.) on the 20th day. The titers were still 1 to 1,600. The third brother (L. M.) revealed the presence of pulmonary tuberculosis and remained at the hospital. Serums continued to be sent at regular intervals. A titer of 1 to 1,600 persisted until the 34th day, then dropped gradually to 1 to 100 by the 70th day, and was completely negative on the 83rd day.

DISCUSSION

This study demonstrates clearly that the results of the meningococcus agglutination test have been consistent over a ten year period, and the test was shown to be of as much value in sporadic cases as it was in those occurring during outbreak periods. The scope of usefulness of the test includes the following: *1*) a diagnostic procedure in cases of clinically typical meningococcic infection but lacking bacteriological proof; *2*) a method of typing the infecting organisms in instances where monovalent typing serum is unavailable; and *3*) tracing the epidemiology of an outbreak.

When used as a diagnostic procedure, serial tests covering the various phases of the disease were found to be of the utmost importance and little or no reliance could be placed on the results obtained from a single specimen. As shown above, both bacteriologically proved and clinically typical cases lacking such proof yielded significant titers in 95 per cent of the instances when tested several times at appropriate intervals. It was also noted in one of our earlier studies (*2*) that serums collected early in the acute phase or late in convalescence were often negative. These observations serve, in large measure, to explain the conflicting results obtained by other investigators (*3–5*).

The use of patients' serum with antigens of known specificity to type the infecting agent is becoming more important due to the discontinuance of the commercial production of typing serums. Furthermore, it may not always be practical to send cultures to typing centers, both because of the hazards involved in the transportation of meningococci and because of the need for isolating the organisms in pure culture for that purpose.

The chief value of tracing the epidemiology of an outbreak by means of the agglutination test is that serums may be withdrawn from comparatively large numbers of individuals and stored in the refrigerator or frozen for future study. This is by far less laborious than isolating the infecting organisms from contacts which involves the availability of special culture media and a large staff, trained in the isolation of these rather fastidious organisms.

For proper interpretation of the test as a diagnostic aid, the results of the agglutination titers must be correlated with the clinical picture. In some instances the presence of significant titers

may be indicative of a latent meningococcic infection which may not necessarily be the cause of the patient's present illness. It is possible that this type of infection accounts for the significant titers observed in approximately 16 per cent of exposures to active meningococcic disease and for the apparent false-positive findings noted in a small percentage of febrile diseases caused by organisms other than the meningococcus. This concept is in accord with Hedrich's recent report (6) in which the first clinical stage of meningococcic disease is described as an asymptomatic nasopharyngeal infection. Some of the false-positive findings may also be due to anamnestic reactions.

This study did not include carriers. But the observations of Rake (7), Miller (3), and Logan (5) indicate that meningococcal agglutinins in low titers may be found in the serum of these individuals. The expectation that a random sampling of serums would show agglutinins in significant titers in a small percentage of instances, and reflect to some degree the carrier frequency, was not borne out, since none of 465 serums sent to the laboratory for syphilis serology yielded a positive agglutination titer.

It was noted in this study that meningococcic agglutinins in significant titers were demonstrated in approximately 23 per cent of our subjects who were infected with other members of the Neisserian group. It is of interest to note that in these subjects agglutinins for Groups 1 and 2-a were observed but none for Group 2. However, the titers in these instances were relatively low and successive specimens were available in only five out of 211 cases. Even in these five individuals, serums were collected at intervals of a month or more, and the progressive changes observed in typical cases of meningococcic infection could not be demonstrated. This aspect of the problem merits further study.

SUMMARY AND CONCLUSIONS

The results of these studies are in agreement with our previous observations. The meningococcus agglutination test has definite value in the establishment of the clinical diagnosis of meningococcic infection in instances where other laboratory methods are inadequate. This applies to sporadic cases as well as to those occurring during outbreaks. In the absence of clinical evidence, it is not possible to distinguish between active and latent infection. The test may be useful in epidemiological studies and in typing the infecting organisms. The importance of performing several tests to demonstrate the change of agglutinin titer cannot be over-emphasized.

ACKNOWLEDGMENTS

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RENAL FUNCTION STUDIES IN TOXEMIA OF PREGNANCY

EXCRETION OF SOLUTES AND RENAL HEMODYNAMICS DURING OSMOTIC DIURESIS IN HYDROPENIA[1]

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The occurrence of proteinuria and the repeated demonstration of depression of filtration rate and renal plasma flow in toxemia of pregnancy are evidence that there is impairment of renal function. Structural abnormalities are not demonstrable in pre-eclampsia although when the stage of eclampsia supervenes morphologic abnormalities are occasionally detectable (1, 2). The invariable presence of edema attests to the fact that the functional lesion involves the mechanism of excretion of water and solutes.

Attempts to define the mechanism of retention of fluid in toxemia of pregnancy have been directed mainly to the hormonal balance of toxemic subjects (3). Investigations focused specifically on the renal mechanism of water and electrolyte excretion and carried out under standard and reproducible conditions have not been reported.

The present investigation was undertaken to apply some of the recently acquired knowledge of the relationship between excretion of water and solutes under the specific conditions of osmotic diuresis in hydropenia to patients with toxemia of pregnancy. The relationship between excreted loads of electrolytes and other urinary constituents, on the one hand, and water on the other, is predictable and reproducible in normal pregnant and non-pregnant subjects studied with this technique (4–6). It has been observed that the amount of water excreted under these conditions is determined solely by the osmotic pressure of the excreted solutes, that the concentrations of sodium and chloride in the urine are remarkably constant and independent of the rate of urine flow, and that the rates of excretion of sodium and chloride are directly proportional to the rate of excretion of the total load of solutes. It was thought that if the technique of osmotic diuresis in hydropenia were applied to cases of toxemia of pregnancy, deviations from the normal pattern might be observed and that these deviations might shed additional light on the nature of the renal defect and the mechanism of edema formation in this disease.

In contrast to the paucity of reports on the renal handling of electrolytes in toxemia, the renal circulation in this disease has undergone much study (7–18). Most of the investigations were carried out after the initiation of therapy, and many either postpartum or after the acute phase of illness had subsided and edema and proteinuria were no longer present. The lack of uniformity of basal conditions in different studies probably accounts for some of the difference of opinion concerning the degree of impairment of renal hemodynamics. Accordingly, the present studies were planned with a view to measuring renal hemodynamics in the acute phase of the illness without the introduction of complicating factors such as therapy or delivery.

The studies were extended to include also cases of essential hypertension in patients in the last trimester of pregnancy. It was thought of interest to apply the aforementioned techniques to cases of this syndrome which resembles to a certain extent the syndrome of toxemia of pregnancy.

MATERIAL AND METHODS

Eight patients with pre-eclampsia, 2 with eclampsia and 4 with essential hypertension associated with pregnancy, were studied. The diagnosis of pre-eclampsia was based on the presence of hypertension, edema and proteinuria after the 24th week of gestation, together with the absence of a history of hypertension prior to pregnancy, and the

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return of the blood pressure to normal levels following delivery. The diagnosis of eclampsia was made when these findings were associated with convulsions and coma. One eclamptic patient (R. K.) had underlying hydronephrosis of the right kidney which required nephrectomy two months after delivery. The four hypertensive patients had persistent hypertension prior to pregnancy. Hypertension was also observed in the early part of pregnancy and was not aggravated during the last trimester. Clinical data on the patients studied are given in Table I. All patients were kept at bed rest from the time of admission until completion of the loading procedure. None of the patients was in labor at the time of study.

Since an attempt was made to investigate their renal function in the acute stage, the patients with pre-eclampsia and essential hypertension received no specific therapy prior to the procedure. The majority underwent the study within 24 hours of admission to the hospital. There was no evidence that any of the patients was experiencing spontaneous diuresis prior to the procedure.

The two eclamptic patients received, on admission, doses of veratrum and magnesium sulfate sufficient to control convulsions and ameliorate their symptoms. After convulsions had been controlled, treatment was temporarily discontinued, and the blood pressure was allowed to return to previous hypertensive levels. In both patients the hypertension, edema and proteinuria were still present during the study.

Osmotic diuresis was induced in hydropenia by administration of hypertonic mannitol solution according to the method previously described (6). Urine was collected during the "resting state" and over two or more periods of 10 to 15 minutes following induction of diuresis. Samples of blood were drawn close to the midpoint of each period. Urine and blood were analyzed for their content of mannitol, para-aminohippurate (PAH), sodium, chloride and potassium according to methods in routine use in these laboratories (4). Effective osmotic activity of the urine was determined by measurement of the freezing point depression.

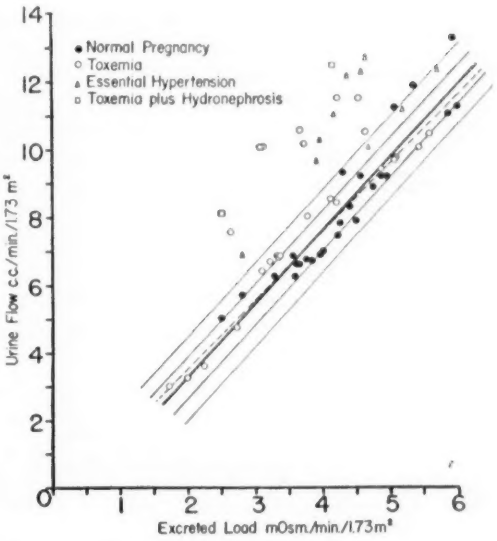

FIG. 1. URINE FLOW VS. EXCRETED LOAD (SEE TEXT)

RESULTS

Figure 1 is a plot of urine flow versus excreted load. The closed circles refer to measurements made on a group of normotensive subjects who were in the last trimester of pregnancy. Some of these data have been reported previously (6) and others were obtained in further studies. Each plotted point refers to a urine collection period. The heavy continuous line is the regression line calculated from the points referring to normal pregnant patients and the two lighter lines represent plus and minus one and two standard er-

TABLE I

Clinical data

Name	Age	Length of gestation	Diagnosis	Blood pressure	Edema	Proteinuria
		weeks		*mm Hg*		
E. W.	31	26	Pre-eclampsia	150/100	+	++
M. J.	20	40	Pre-eclampsia	140/95	+	+
M. L.	23	30	Pre-eclampsia	160/110	+++	++++
I. M.	21	36	Pre-eclampsia	170/110	+	++
A. P.	41	32	Pre-eclampsia	185/114	++	+
B. M.	36	37	Pre-eclampsia	174/115	++	++
A. E.	15	39	Pre-eclampsia	160/110	++++	++++
A. B.	18	36	Pre-eclampsia	164/114	++	+++
R. K.	28	34	Eclampsia and hydronephrosis	180/120	++	++++
E. C.	32	40	Eclampsia	180/110	++	+
S. W.	38	32	Essential hypertension	190/120	0	0
R. B.	36	28	Essential hypertension	184/110	0	++
O. W.	27	34	Essential hypertension	160/105	0	0
E. R.	39	34	Essential hypertension	190/120	0	0

rors of the estimate of the regression equation. The open circles refer to urine collection periods from patients with toxemia and the open triangles to urine collections from patients with essential hypertension in pregnancy. The interrupted line is the regression line from the data of Brodsky (5) who subjected 16 normal non-pregnant individuals to osmotic diuresis under conditions identical with those described in this paper. There is a close correlation between the relationship of flow and load in the two groups of normal cases suggesting that patients with normal pregnancy do not have any abnormality in their ability to conserve water under hydropenic conditions.

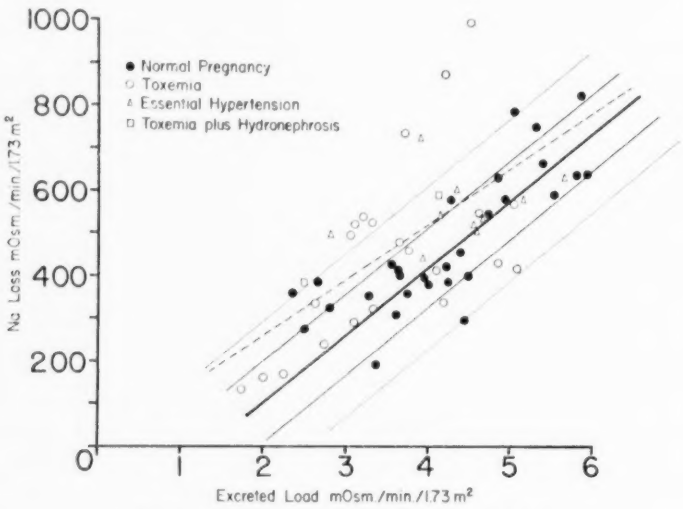

FIG. 2. SODIUM EXCRETION VS. EXCRETED LOAD

The three open circles falling well to the left of the line indicating ± 2 S.E. refer to a single patient with toxemia of pregnancy. All other points fall either between or close to the lines indicating ± 2 S.E. (See text.)

NOTE: The ordinate of this figure should read *μOsm./min./1.73 m²* instead of mOsm./min./1.73 m², as it now appears.—Author.

The open circles and triangles refer to measurements obtained in cases of toxemia and essential hypertension in pregnancy. It is evident that any point falling to the left of the normal line indicates that the given load requires or has available a greater than normal quantity of water for its excretion. The majority of points referring to cases of abnormal pregnancy fall outside the line demarcating 2 S.E. of the estimate indicating that there is a significant difference between the normal and abnormal groups in terms of excretion of water per unit of solute load.

Figure 2 is a plot of Na loss *vs.* excreted load. The symbols are the same as in Figure 1. Once more the regression line, plus and minus one and two standard errors of the estimate for the normotensive pregnant patients has been included as well as the regression line calculated from the data of Brodsky (5) for normotensive non-pregnant patients. The fact that the regression line for the normal non-pregnant patients falls outside the line representing one standard error of the estimate for the normotensive pregnant patients would seem to indicate that in normal pregnancy there is some tendency for lower rates of excretion of Na per unit of total excreted load, but the significance of this observation is not apparent. The majority of points for the abnormal cases do not deviate widely from the regression lines calculated from the data of the normal non-pregnant and pregnant subjects, indicating that there is no material difference between the rate of loss of Na per unit of total solutes excreted in normal cases and cases of toxemia or hypertension. The rela-

TABLE II

Mannitol and PAH clearances and filtration fractions of hypertensive and toxemic cases

Patient*	Diagnosis	Mannitol clearance	PAH clearance	Filtration fraction
		c./min./1.73 m.²	*cc./min./1.73 m.²*	
E. W.	Toxemia	57	441	0.13
E. C.	Toxemia	101	427	0.24
M. J.	Toxemia	100	725	0.14
M. L.	Toxemia	89	749	0.13
I. M.	Toxemia	76	443	0.17
A. P.	Toxemia	70	699	0.10
B. M.	Toxemia	55	402	0.14
A. E.	Toxemia	84	502	0.17
A. B.	Toxemia	60	623	0.10
S. W.	Essential hyperten.	65	410	0.16
R. B.	Essential hyperten.	62	292	0.21
O. W.	Essential hyperten.	95	420	0.23
E. R.	Essential hyperten.	95	564	0.17
Mean of 9 patients with toxemia		77	557	0.144
Mean of 4 patients with essential hypertension		79	422	0.191
Mean of 7 normal pregnancies		109	699	0.157

* Patient R. K. was not included because she had underlying hydronephrosis. Her mannitol clearance was 33 and her PAH clearance was 236 cc./min./1.73 m².

tionship of chloride loss to excreted load resembled closely that for sodium.

Table II presents the mannitol and PAH clearances and filtration fractions of the two groups of abnormal pregnancies, comprising nine cases of toxemia and four of essential hypertension. (R. K., who had eclampsia with underlying hydronephrosis, is excluded.) Each figure is the average of 2 or 3 urine collection periods. The means of the clearances of mannitol and PAH for each group were reduced when compared with the means for the group of seven cases of normal pregnancy. Calculation of the significance of the differences between these means has been made by computating Student's t for each pair of values according to the method of Fisher (19). GFR was significantly reduced as compared with the normal both in the toxemic ($p = 0.002$) and in the hypertensive ($p = 0.018$) group; RPF was also reduced in both groups ($p = 0.054$ and $p = 0.008$, respectively). It was not possible to demonstrate any significant difference between the group with essential hypertension and that with toxemia.

Table III presents renal vascular resistances

TABLE III

Renal vascular resistances in cases with toxemia and hypertension

Values are given in dynes sec. cm.$^{-5}$

Patient	Diagnosis	Total renal resistance	Afferent resistance	Efferent resistance	Venular resistance
E. W.	Toxemia	8,181	4,267	1,223	2,691
E. C.	Toxemia	13,869	9,332	2,217	2,320
M. J.	Toxemia	6,813	3,953	1,283	1,577
M. L.	Toxemia	7,459	4,834	1,105	1,519
I. M.	Toxemia	10,469	6,713	1,534	2,231
A. P.	Toxemia	10,100	6,971	1,143	1,986
B. M.	Toxemia	13,924	9,748	1,260	2,917
A. E.	Toxemia	13,499	8,895	1,870	2,734
A. B.	Toxemia	8,373	5,289	1,010	2,075
S. W.	Ess. hyp.	16,408	11,929	1,567	2,912
R. B.	Ess. hyp.	19,920	13,633	1,942	4,345
O. W.	Ess. hyp.	12,922	8,166	2,091	2,665
E. L.	Ess. hyp.	11,368	7,763	1,572	2,033
Mean of 9 patients with toxemia		10,229	6,666	1,405	2,228
Mean of 4 patients with ess. hyp.		15,154	10,397	1,793	2,989
Mean of 7 normal pregnancies		5,178	1,883	1,531	1,751

calculated from the values for the mean arterial blood pressure, RBF, GFR, hematocrit, and total serum proteins, according to the formulae of Gomez (20). The increase in total renal resistance in the toxemic patients as compared with the normal pregnant patients was highly significant ($p = 0.0007$). The major increase in resistance appeared to be in the afferent segment ($p = 0.0001$) although a significant increase occurred also in venular resistance ($p = 0.027$). In essential hypertension total renal resistance was also greatly increased as compared with the normal ($p = 0.0002$) and once again the afferent segment appeared to be the site of largest increase in resistance.

Tables IV, V, and VI present data obtained from three patients, one with eclampsia, one with pre-eclampsia, and one with essential hypertension. Each of the 3 cases is representative of the groups studied. In Table V it will be seen that

TABLE IV

Blood pressure, urine flow, renal plasma flow, glomerular filtration rate, filtration fraction, effective osmotic activity, urine concentration of Na, Cl and potassium, and total solute loss during osmotic diuresis with mannitol

All flows are calculated per minute per 1.73 m.²

Patient: E. C., Weight: 86 kg., S.A.: 2.01 m.², Age: 30, Diagnosis: Eclampsia

Period	Concurrent time	Mean B.P.	Urine flow	PAH clearance	Mannitol clearance	F.F.	Na	Cl	K	Effective osmotic activity	Total solutes
	min	*mm. Hg*	*cc.*	*cc.*	*cc.*		*mEq./L.*	*mEq./L.*	*mEq./L.*	*mOsm./L.*	*μOsm./min.*
Prelim	−30 to 0	155/112	0.78				143	123	12	890	690
	0 to 10	I.V. injection of 310 cc. 25% mannitol, 5.16 cc. 20% NaPAH									
	10 to 56	I.V. injection of 25% mannitol at 4.64 cc./min., 20% NaPAH at 0.13 cc./min.									
1	26 to 36	173/121	9.82	499	105	0.21	43	43	14	517	5,077
2	36 to 48	181/119	9.47	402	99	0.25	45	48	12	512	4,849
3	48 to 56	200/110	9.73	381	99	0.26	58	51	10	518	5,040

TABLE V

Blood pressure, urine flow, renal plasma flow, glomerular filtration rate, filtration fraction, effective osmotic activity, urine concentration of Na, Cl and potassium, and total solute loss during osmotic diuresis with mannitol

All flows are calculated per minute per 1.73 m.²

Patient: I. M., Weight: 61 kg., S.A.: 1.64 m.², Diagnosis: Pre-eclampsia

Period	Concurrent time	Mean B.P.	Urine flow	PAH clearance	Mannitol clearance	F.F.	Na	Cl	K	Effective osmotic activity	Total solutes
	min.	*mm. Hg*	*cc*	*cc.*	*cc.*		*mEq./L.*	*mEq./L.*	*mEq./L.*	*mOsm./L.*	*μOsm /min.*
Prelim.	−30 to 0	165/112	0.21				84	111		812	172
	0 to 10	I.V. injection of 222 cc. 25% mannitol, 3.66 cc. 20% NaPAH									
	10 to 94	I.V. injection of 25% mannitol at 3.33 cc./min., 20% NaPAH at 0.09 cc./min.									
1	37 to 48	166/112	6.83	459	81	0.18	47	41		490	3,347
2	48 to 61	160/113	6.45	425	70	0.16	45	42		480	3,096

TABLE VI

Blood pressure, urine flow, renal plasma flow, glomerular filtration rate, filtration fraction, effective osmotic activity, urine concentration of Na, Cl and potassium, and total solute loss during osmotic diuresis with mannitol

All flows are calculated per minute per 1.73 m.²

Patient: R. B., Weight: 52 kg., S.A.: 1.48 m.², Diagnosis: Essential hypertension

Period	Concurrent time	Mean B.P.	Urine flow	PAH clearance	Mannitol clearance	F.F.	Na	Cl	K	Effective osmotic activity	Total solutes
	min.	*mm. Hg*	*cc.*	*cc.*	*cc.*		*mEq./L.*	*mEq./L.*	*mEq./L.*	*mOsm./L.*	*μOsm./min.*
Prelim.	−30 to 0	148/104	0.02				52	233	75	914	18
	0 to 10	I.V. injection of 190 cc. 25% mannitol, 3.12 cc. 20% NaPAH									
	10 to 82	I.V. injection of 25% mannitol at 2.85 cc./min., 20% NaPAH at 0.08 cc./min.									
1	26 to 37	156/108	6.91	258	54	0.21	72	48	7	407	2,812
2	37 to 48	160/110	9.68	325	70	0.22	75	61	6	402	3,891

during the study there was a rise in the systolic blood pressure without a significant change in the diastolic blood pressure. It seems unlikely that this rise in blood pressure was due to intravenous injection of mannitol since increase of comparable magnitude did not occur in other patients studied.

DISCUSSION

Of particular interest is the failure to demonstrate in toxemia of pregnancy a consistent abnormality in the renal handling of sodium and chloride during osmotic diuresis with a nonelectrolyte. During mannitol diuresis induced in the hydropenic state in normal individuals the concentrations of sodium and chloride in the urine are relatively constant irrespective of the rate of urine flow (5, 21). The constancy of concentration is thought to reflect the maximum concentration difference which the proximal tubule is capable of achieving between plasma and tubular fluid. However, the concentration difference may be altered by varying the physiological conditions of the experiment, such as loading with sodium salts of various inorganic acids (22), section of the splanchnic nerve (23), or infusion of epinephrine (24). Tarail (25) induced osmotic diuresis in patients with edema of congestive heart failure and cirrhosis of the liver, and demonstrated that the losses of Na per unit of excreted glucose were considerably smaller than the losses which occurred in the same patients during recovery from the edematous state. Recalculation of their data showed that the difference in Na concentration between proximal tubular fluid and plasma was considerably greater while edema was present than was observed in the same subjects when free of edema. The reason for the lack of agreement between their observations and those presented here are not apparent. It may be that the mechanism of edema formation varies with differing underlying etiological factors or that the patients studied by Tarail and his associates (25), and these reported here, were in different stages of edema formation. The absence of a defect in the concentrating mechanism for sodium in toxemia of pregnancy does not absolve the kidneys from being at fault in the accumulation of edema—rather it indicates that the retention of sodium and chloride is not associated with the ability of the proximal nephron to abstract these ions from the proximal tubular fluid against a greater concentration difference than that which is achieved in normal subjects.

It is not possible to determine with absolute certainty whether deviation from the normal relationship between flow and load observed in the edematous toxemic subjects was due to a concentration defect or to the greater availability of water for excretion. The patients with essential hypertension were no more edematous than their counterparts with normal blood pressure, so it seems unlikely that the larger volume of water excreted by them could be attributed to the availability of excessive quantities of water. In the cases of toxemia, on the other hand, marked edema was usually present and it is conceivable that edema fluid was available to the kidneys for excretion with the consequence that significant deviations from the normal flow-load relationship occurred. However, since some patients with marked edema showed no deviation from the normal response there is no reason to believe that the mere presence of edema results in the availability of additional water for excretion. Reference to Tables IV and V, which present data obtained in studies on edematous patients, reveals that in neither of these subjects was there any abnormality in the water conservation mechanism. On the other hand, it is possible that additional water is available for excretion only during spontaneous diuresis. As has been pointed out above, all patients were studied as soon after admission as possible and it is probable that none was experiencing spontaneous diuresis during the loading procedure. We believe, therefore, that the abnormality in flow-load relationship is indicative of a concentration defect in these subjects.

Studies of renal hemodynamics in toxemia of pregnancy have indicated that in the majority of cases glomerular filtration rate is depressed (8–16). It was pointed out, however, that most of these investigations were carried out after treatment had been instituted and many after the acute phase of illness had subsided. The present data, obtained before institution of specific therapy in all but two instances, are in general agreement with the above studies. Renal blood flow has been found by some investigators to be normal or only slightly depressed in the majority of cases (8, 10, 17, 18), and by others to be more markedly de-

pressed (9, 11, 15, 16). The present study shows that in the acute state the mean values for renal plasma flow in toxemic subjects is reduced when compared with normal pregnant subjects in a comparable stage of gestation, but the reduction is less significant than is the reduction of filtration rate.

Reports of renal hemodynamics in cases of essential hypertension in pregnancy are less numerous. It has been found that renal plasma flow is reduced without much alteration in glomerular filtration rate (8, 16). In the present study the values for both renal plasma flow and glomerular filtration rate were significantly depressed in comparison with the figures for the normal pregnant patients. The depression was more marked in renal plasma flow than in glomerular filtration rate.

Calculation of renal vascular resistances using the formulae of Gomez (20) is based on the assumption that glomerular membrane permeability is not impaired. It has been claimed by Bell (26) that in eclampsia there may be an increase in the thickness of the glomerular basement membrane. Sheehan (1) and others (2) believe that this pathologic change does not occur in pre-eclampsia and is rarely encountered in eclampsia. Thus it is probable that the values assumed for the gross permeability coefficient in all cases, with the possible exception of the two with eclampsia, are correct. Another source of error in calculation of renal resistance is the presence of proteinuria. However, if it is assumed that all protein in the urine is filtered at the glomerulus, simple calculation shows that with the usual amount of protein lost in the urine in toxemia or essential hypertension (1 to 4 grams per 24 hours) the concentration of protein in the glomerular filtrate does not exceed 20 mgm. per 100 cc. The oncotic pressure and change in viscosity exerted by this minute amount of protein is negligibly small and probably introduces no major error in the calculation of the distribution of resistance to blood flow in the kidney. Finally venous pressure has been shown to be normal in toxemia (27) so that the assignment of the usual value to *Pv.* in Gomez' formula probably introduces no error. The calculations presented in this report indicate that in toxemia and essential hypertension associated with pregnancy the major resistance to the flow of blood is in the afferent segment. Kenney (15) arrived at similar conclusions although his methods were different from those presented here. The values obtained here in cases of essential hypertension in pregnancy are in agreement with the data of Gomez (20) who found that the major site of increased resistance in non-pregnant subjects with essential hypertension was in the afferent segment.

SUMMARY AND CONCLUSIONS

1. A study of excretion of electrolytes and water and renal hemodynamics during osmotic diuresis with mannitol in hydropenia was carried out on 8 patients with pre-eclampsia, 2 with eclampsia and 4 with essential hypertension associated with pregnancy.

2. It was not possible to demonstrate any abnormality in the renal handling of Na and Cl in cases of abnormal pregnancy.

3. Impairment of conservation of water per unit of excreted solute load was present in several cases of hypertension and toxemia.

4. Renal plasma flow (PAH clearance) and glomerular filtration rate (mannitol clearance) were reduced as compared to values obtained on normal subjects in the same stage of pregnancy.

5. Renal vascular resistance was increased in the abnormal cases mainly as a result of increase of the afferent segment resistance.

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THE ARTERIAL BLOOD GASES, THE OXYGEN DISSOCIATION CURVE, AND THE ACID-BASE BALANCE IN POLYCYTHEMIA VERA[1]

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Studies of the circulation and pulmonary function in normal children and in patients with polycythemia related to cyanosis in congenital heart disease raised questions concerning the extent to which polycythemia itself might be responsible for some of the abnormalities observed in the blood in cyanotic congenital heart disease. These questions concerned the possibility that the increased viscosity of polycythemic blood might prevent proper diffusion of oxygen in the alveolar-capillary system, resulting in arterial oxygen unsaturation, the characteristics of the oxyhemoglobin dissociation curve in polycythemia vera, since this has been found shifted to the right in the blood of patients with cyanotic congenital heart disease (1), and the relation of a low CO_2 content and combining power to polycythemia, since these were observed to be low in the secondary polycythemia of congenital heart disease (2–4).

Studies of the blood in polycythemia vera were made and compared with similar studies in cyanotic congenital heart disease. These included determinations of the oxygen content, oxygen capacity and oxygen saturation of arterial blood, the effect of breathing oxygen on the oxygen content of such blood, the oxyhemoglobin dissociation curve and the carbon dioxide content, combining power and tension, and the pH_a of arterial blood. It was hoped that some of these might be sufficiently characteristic to be helpful in differentiating primary from secondary polycythemia. This report presents the data obtained during these studies.

MATERIAL

Blood from patients considered to have polycythemia vera was made available by the Department of Hematology.[2] In each case the patient rested one-half hour before the arterial sample was drawn. The patients varied in age from 14 to 62 years. For purposes of comparison the arterial blood from a group of 9 medical students was investigated in the same manner.

METHODS

Samples of arterial blood were collected in a syringe containing mineral oil to fill the dead space of the syringe. The blood was transferred immediately to a sampling tube containing mercury and a small amount of heparin, the tube was sealed with mercury, rotated to mix the blood with heparin, and stored in ice water in a refrigerator until the analyses were completed.

For the determination of oxygen capacity and carbon dioxide combining power, 4 to 5 ml. samples were equilibrated at 37° in a tonometer, of approximately 225 ml. capacity, which contained carbon dioxide and oxygen at approximate tensions of 40 mm. and 185 mm., respectively. The oxygen and carbon dioxide contents of both the samples as drawn and those equilibrated in the tonometers were determined by the manometric method of Van Slyke and Neill (5). From these data and the gas contents of the tonometers, determined by Haldane analysis, the percentage of oxygen saturation, carbon dioxide combining power (T_{40}) of the blood and plasma, the carbon dioxide tension, and the pH_a were calculated by methods described by Dill and his coworkers (6).

The *in vitro* tonometer method was employed for the determination of the oxygen dissociation curve, essentially as described by Dill in the study of Keys, Hall, and Barron (7). Further details are given in an earlier report (8).

Oxygen for breathing was supplied from a Douglas bag through a modified Henderson-Haggard valve (9) and an anesthesia mask. An arterial sample was drawn just before oxygen breathing began and after it had continued for 5 minutes.

RESULTS

Table I[3] gives the results of analyses of samples of arterial blood from 17 patients with poly-

[1] Supported by a grant from the Douglas Smith Foundation for Medical Research at the University of Chicago.

[2] Through the courtesy and cooperation of Dr. Leon Jacobson, Dr. Matthew Block and others in the department.

[3] The patients studied had polycythemia of unknown origin, without demonstrable cardiac or pulmonary disease,

cythemia vera and from 9 young adult males who were in good health. Since the same methods were employed by Dill, Edwards and Consolazio (10) in a study of 12 normal adult males, the mean values and the range of variation observed in their study have been included in the table for reference. Aside from slightly higher values for oxyhemoglobin capacity in our group of normal males and the resultant effect of the increased ratio of red cells to plasma on the carbon dioxide

with one exception. Of the three with low arterial oxygen saturation, at autopsy J. P. revealed no congenital cardiac defects or primary pulmonary disease. M. H. had no cardiac enlargement and had clear lung fields, although he had a slow auricular fibrillation thought to be arteriosclerotic in origin. Persistent polycythemia, controlled with nitrogen mustard and P^{32}, hyperplastic bone marrow, and leucocytosis varying from 8,000 to 17,000 before therapy were considered diagnostic of a primary polycythemia and this diagnosis was never questioned. J. D., whose polycythemia is controlled by repeated phlebotomy, showed no change in blood gases after a four year interval. She had, however, thoracolumbar scoliosis, maximal at T 12, and occasional episodes of pulmonary hemorrhage during periods of marked polycythemia. Polycythemia and cyanosis had been apparent when first seen in 1939, when the lung fields were clear. Pulmonary function studies in 1952 are probably complicated by residual effects of hemorrhage. The lung volumes were: inspiratory capacity 1,275 cc., expiratory reserve 577 cc., residual volume 1,004 cc., or a total capacity of 2,856 and vital capacity of 1,852. The inspiratory capacity is low. The alveolar-arterial gradients were greatly increased, the pCO_2 gradient being 16.4 mm. and the pO_2 gradient 66.5 mm. The early diagnosis was polycythemia vera with concurrent scoliosis, and she was treated with phenylhydrazine, x-ray therapy, and for the past few years with repeated phlebotomy.

Table I

Results of gas analysis of arterial blood from patients with polycythemia and from normal young male adults

Subject	Age	Sex	Oxyhemoglobin capacity	Oxyhemoglobin content	Percentage saturation	Blood CO_2 T_{40}	Plasma CO_2 T_{40}	CO_2 Tension	pH_a
					Patients with Polycythemia Vera				
P. G.	14	M	21.1	19.0	90.0	45.1	55.1	30.1	7.47
J. P.	14	F	26.9	21.7	80.5	39.1	51.4	30.2	7.44
J. B.	21	M	23.0	22.2	95.7	44.0	54.8	41.2	7.37
G. T.	25	M	23.1	20.7	89.5	45.3	56.4	39.0	7.41
R. D.	26	M	34.4	32.5	94.5	37.5	53.2	36.8	7.40
D. J.	36	F	29.6	23.6	79.5	42.3	56.8	39.9	7.42
A. H.	51	F	27.7	27.1	97.6	37.9	49.5	33.8	7.38
M. N.	55	F	31.1	29.3	94.4	40.1	54.3	38.8	7.39
K. B.	40	F	19.3	18.8	97.6	46.0	54.8	37.1	7.39
A. M.	59	M	25.9	24.3	93.8	45.1	57.8	50.6	7.34
C. G.	54	M	25.5	24.8	97.1	42.7	54.6	41.0	7.37
J. K.	60	M	31.8	29.4	92.6	38.5	52.5	45.9	7.33
W. K.	61	F	25.0	22.9	91.6	42.8	54.3	41.2	7.37
M. H.	60	M	28.5	24.5	86.1	42.7	57.1	40.3	7.41
C. E.	52	M	32.6	31.2	95.7	41.1	56.3	41.2	7.39
F. N.	47	M	25.2	25.2	100.0	45.0	57.1	39.4	7.40
S. B.	62	M	28.0	26.6	95.0	39.5	51.8	41.1	7.35
Mean			27.0 ± 1.0	24.9 ± 1.0	92.4 ± 1.4	42.0 ± 0.4	54.6 ± 0.6	39.3 ± 1.2	7.39 ± 0.01
					Normal Young Adults				
M. S.	33	M	22.1	21.4	96.6	46.0	56.7	41.9	7.38
J. T.	27	M	22.3	21.2	94.9	46.5	57.4	37.6	7.42
A. M.	22	M	20.9	20.0	95.6	48.1	58.5	42.4	7.39
G. M.	22	M	22.5	21.6	95.8	46.8	58.0	43.1	7.40
W. F.	30	M	19.4	18.9	97.2	49.5	59.4	39.5	7.42
N. G.	25	M	21.0	20.2	96.1	46.8	57.0	34.9	7.44
R. S.	22	M	21.7	20.4	94.0	47.5	58.2	41.3	7.40
H. P.	22	M	19.8	18.9	95.4	49.3	59.3	43.3	7.39
J. R.	20	M	20.8	20.2	97.3	48.9	59.5	46.1	7.38
Mean			21.2 ± 0.4	20.3 ± 0.3	95.9 ± 0.4	47.7 ± 0.7	58.2 ± 0.4	41.1 ± 1.1	7.40 ± 0.01
Mean, Dill's group			19.9	19.0	95.5	48.2	58.2	41.0	7.40
12 adult males			(17.3-21.7)	(16.5-20.6)	(94.2-96.0)	(44.2-50.6)	(53.8-61.4)	(37.6-42.5)	(7.35-7.44)

content of the blood, the results of the two studies on normal males are very similar.

Arterial saturations in normal adults were found in the present study to vary from 94.0 to 97.5 per cent, with a mean of 95.9 per cent. The lower limit of arterial saturation as determined by the tonometer method appears to be near 94 per cent. These values are lower than the mean value of 97.9 per cent saturation which Wood (11) found for 46 arterial samples from normal subjects when he employed the method described by Roughton, Darling, and Root (12). Roughton has shown that there is a systematic error of approximately 2 per cent when the tonometer method is used for determining oxygen capacity. If a correction of 2 per cent is subtracted from the oxyhemoglobin capacities of the present data, a mean arterial saturation of 97.9 per cent is obtained for the normal adult males. Since the tonometer method has shown such good agreement in the results of two laboratories, confidence can be placed in the comparison of results from the normal group with those from patients with polycythemia vera, regardless of whether the absolute value is correct.

In the patients with polycythemia vera, eight patients were found to have arterial saturations less than 94 per cent, and four of these had saturations less than 90 per cent. One of the patients with a low saturation, case 2, died of an intercurrent infection, a brain abscess, 3 years subsequent to the determinations. No cardiac or pulmonary pathology was identified on autopsy and the diagnosis of polycythemia vera was confirmed.

Five patients were studied before and after reduction of polycythemia by therapy. The results of analysis of arterial blood from these patients are given in Table II. It is evident that the arterial saturation shows no consistent response. In one patient with an initial oxygen capacity of 30 vollumes per cent and arterial saturation of 79 per cent, practically no change occurred when the oxygen capacity was reduced to 24 volumes per cent. In another patient arterial saturation was initially high and remained high when the oxygen capacity was reduced from 28 to 21 volumes per cent. In two cases low initial arterial saturations of 89 and 86 per cent were raised to 98 and 94 per cent, respectively, after polycythemia had been reduced; but in another instance a saturation of 94 per cent was reduced to 89 per cent when the oxygen capacity was reduced from 31 to 21 volumes per cent. The variation in response of the arterial saturation to reduction of the polycythemia is confusing. It suggests that factors other than polycythemia itself are concerned in the maintenance of arterial saturation.

In the hope of obtaining information concerning the cause of arterial unsaturation observed in an occasional patient with polycythemia vera, the effect of breathing 99.6 per cent oxygen upon the arterial saturation was tested in 9 patients. In this group arterial saturation while breathing air ranged from 76.9 to 97.1 per cent. This group included four patieints with saturations below the normal range. Table III gives the results both of the patients with polycythemia vera and a group of healthy young adult males. A greater increase in oxygen content due to oxygen breathing is found in the polycythemic group. It is related

Table II

Effects of reduction therapy upon the oxygen saturation and the acid-base equilibrium of the blood in patients with polycythemia vera

Subject	Age	Sex	Oxyhemoglobin capacity		Oxyhemoglobin content		Percentage saturation		Blood CO_2 T_{40}		Plasma CO_2 T_{40}		CO_2 Tension		pH_e	
			Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
M. N.	55	F	31.1	21.1	29.3	18.9	94.4	89.4	40.1	46.9	54.3	57.3	38.8	40.4	7.39	7.40
D. J.	36	F	29.6	24.4	23.6	18.6	79.5	77.7	42.3	43.6	56.8	54.7	39.9	46.2	7.42	7.35
A. H.	51	F	27.7	21.5	27.1	20.8	97.6	96.8	37.9	44.4	49.5	54.6	33.8	40.0	7.38	7.38
G. T.	25	M	23.1	21.9	20.7	21.5	89.5	98.0	45.3	44.7	56.4	55.0	39.0	35.7	7.41	7.42
M. H.	60	M	28.5	21.6	24.6	20.2	86.1	93.6	42.7	47.9	57.1	58.7	40.3	37.6	7.41	7.43

Table III

Effect of breathing oxygen on the oxygen content of arterial blood in patients with polycythemia vera and in normal young adults

Subject	Arterial saturation, breathing air	Oxygen Content of the Blood			Oxyhemoglobin capacity	Physically dissolved oxygen
		Breathing air	Breathing oxygen	Δ		
		Patients with Polycythemia Vera				
P. C.	90. 0	19. 22	21. 61	2. 39	21. 09	0. 52
M. N.	89. 4	19. 08	21. 41	2. 33	21. 13	0. 28
R. D.	94. 5	32. 67	35. 93	3. 26	34. 39	1. 54
W. K.	91. 6	23. 14	26. 47	3. 33	25. 04	1. 43
S. B.	95. 0	26. 78	29. 06	2. 28	27. 98	1. 08
J. B.	95. 7	22. 22	24. 43	2. 21	23. 01	1. 42
A. M.	93. 8	24. 48	27. 47	2. 99	25. 89	1. 41
C. G.	97. 1	24. 95	26. 92	1. 97	25. 49	1. 43
D. J.	76. 9	15. 79	21. 07	5. 28	20. 30	0. 77
		Normal Young Adults				
M. S.	96. 6	21. 56	22. 98	1. 42	22. 13	0. 85
J. T.	94. 9	21. 39	23. 55	2. 16	22. 34	1. 21
A. M.	95. 6	20. 15	21. 94	1. 79	20. 89	1. 05
G. M.	95. 8	21. 53	23. 79	2. 26	22. 75	1. 04
W. F.	97. 2	19. 09	20. 58	1. 49	19. 44	1. 14
N. G.	96. 1	20. 39	21. 47	1. 08	21. 03	0. 44
R. S.	94. 0	20. 55	22. 33	1. 78	21. 66	0. 67
H. P.	95. 4	19. 05	20. 96	1. 91	19. 77	1. 19
J. R.	97. 3	20. 42	21. 70	1. 28	20. 79	0. 91

to their lower mean arterial saturation and their higher oxygen capacity. In both groups physically dissolved oxygen as measured by the oxygen content in excess of the hemoglobin capacity is in every instance lower than the theoretical amount, which is approximately 2 ml. per 100 ml. This is calculated from the solubility coefficient of oxygen in blood as given by Sendroy, Dillon, and Van Slyke (13) and the alveolar oxygen tension. The discrepancy may be due to any number of factors described by Wood (11) with the additional possibility that some of the dissolved oxygen, held in solution by the high oxygen pressure, may be lost by reduction in pressure during the handling involved in transferring the blood from the syringe to the sampling tube and thence into the measuring pipette. Since the methods employed were the same for both the normal group and the patients with polycythemia vera, the finding of comparable values for physically dissolved oxygen in the two groups may be explained by the observation of Sendroy and his co-workers (13) that the solubility coefficient of oxygen in blood increases with increasing hemoglobin capacity.

To determine whether the hemoglobin of polycythemic blood differs from normal blood in its affinity for oxygen, the oxyhemoglobin dissociation curve of the blood was determined for 8 patients with polycythemia vera. The results are shown in Figure 1 as percentage saturation plotted against corresponding tensions, for oxygen tensions of 30, 40, 50, 60, 70, and 80 mm. The solid line of the chart represents the oxyhemoglobin dissociation curve for normal adults which was determined in our laboratory by the same methods (8). The area within the broken lines represents the range of variation for normal adults as defined by plus or minus 2 standard deviations from the mean. There is no evidence that the oxygen dissociation curve of the blood from patients with

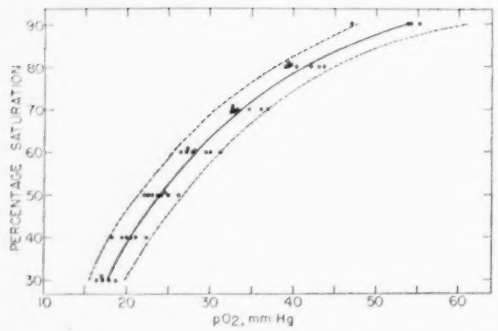

FIG. 1. RELATION BETWEEN OXYGEN TENSION AND PERCENTAGE OF OXYGEN SATURATION IN PATIENTS WITH POLYCYTHEMIA VERA

The curve (solid line) drawn through the plotted points connects mean values of oxygen saturation at corresponding oxygen tensions for normal adults (8). The probable area of normal variation is included between the broken lines which connect points which are ± two standard deviations from the mean.

polycythemia vera differs from the normal. Unfortunately the number of cases is comparatively small but includes three with arterial saturation below the normal limit.

In contrast to the polycythemic blood in cyanotic congenital heart disease, there is little evidence of abnormality in the acid-base equilibrium of the blood in patients with polycythemia vera. The few abnormalities that are apparent in Table III appear to be isolated and not characteristic of the group as a whole. In general, carbon dioxide content and combining power of the blood tend to be lower than normal. This can be attributed to the greater percentage of cells in polycythemic blood since the carbon dioxide concentration is lower in red cells than it is in the plasma. In contrast to whole blood, the carbon dioxide combining power of the plasma is within the normal range in all except four cases. In 3 patients the arterial carbon dioxide tension appears lower than normal and in 2 instances higher. The pH_a is slightly below the lower limit of normal in 3 patients.

DISCUSSION

In the absence of specific diagnostic criteria, polycythemia vera frequently is a diagnosis by exclusion of known causes of polycythemia. In addition to polycythemia related to congenital heart disease and pulmonary disease, abnormal blood pigments may contribute to the production of cyanosis and polycythemia. Methemoglobin may change to oxyhemoglobin during equilibration with oxygen in the determination of hemoglobin capacity. Since the oxygen content has not changed, the saturation may be low. Direct spectrophotometric analysis for methemoglobin was not done in the patients studied, but none had received any medication contributing to methemoglobin formation. The position of the oxygen dissociation curves within the normal range of adults offers indirect evidence for the absence of methemoglobin, since Darling and Roughton (14) have shown that in mixtures of methemoglobin and hemoglobin the oxygen dissociation curve is shifted to the left. The curve of D. J. who had arterial saturations as low as 79 per cent lay farther to the right than that of any of the polycythemia patients studied.

The statement is frequently made that the resting arterial saturation is normal in polycythemia vera in contrast to a lower saturation in secondary polycythemia. This conclusion has been affirmed by Moschcowitz (15), Hitzenberger (16), Brooks (17), Mohr (18), Röver (19), and more recently by Wasserman, Dobson, and Lawrence (20), who state that there is no great deficiency in the saturation of the circulating hemoglobin with oxygen. However, more recently Newman, Feltman, and Devlin (21) reported low saturation in 2 of 5 patients studied.

The data of Table I are also at variance with the commonly accepted belief that while arterial saturation in polycythemia vera tends to fall in the lower range of normal, it is within the normal range. The few low saturations which have been reported have been explained on the basis of probable pulmonary complications. Although a few of the patients included in the study may possibly have had complicating abnormalities of pulmonary origin, these could not be identified by clinical or x-ray examination, except in one instance where there was scoliosis, and pulmonary hemorrhages at the height of polycythemia. In the case of J. P., 14 years old, who had an arterial saturation of 80.5 per cent the diagnosis of polycythemia vera was confirmed by autopsy 3 years subsequent to the blood studies, when she died from a brain abscess. If normal arterial oxygen saturation is a required criterion for the diagnosis of polycythemia vera,

a number of polycythemias of unknown origin that have diminished arterial oxygen saturation will remain.

In our data the lower than normal saturations are not associated with the higher oxyhemoglobin capacities. On the other hand, of the 4 cases with the most marked polycythemia, with oxyhemoglobin capacities of 31.1 to 34.4 volumes per cent, only one had a slightly reduced saturation of 92.6 per cent and the others were well within the normal range. The high viscosity of the blood in polycythemia does not in itself prevent normal saturation of the blood with oxygen as it passes through the pulmonary capillaries.

The same variability and lack of correlation between polycythemia and arterial unsaturation is seen, in a converse sense, in cyanotic congenital heart disease. Many infants are seen with arterial saturations of 50 to 80 per cent, but with hemoglobin concentrations as low as 10 gms. per 100 ml. Older children with cyanotic congenital heart disease may not necessarily have a compensatory polycythemia.

The results with oxygen breathing prove that any arterial unsaturation observed while the patient with polycythemia is breathing air cannot be attributed to a venous admixture greater than may occur in normal persons. This would almost certainly place the source of the unsaturation in the lungs, either in poorly ventilated areas which are perfused with blood, or in a change in the alveolar membrane which impairs diffusion of oxygen from the alveoli to the blood. Reports in the literature suggest that both of these sources of unsaturation are possible. Harrop and Heath (22) found that the rate of diffusion of carbon monoxide across the alveolar walls is lower in patients with polycythemia vera than in normal persons, and that patients who have normal arterial saturation at rest may have this saturation reduced when they engage in exercise. Newman, Feltman, and Devlin (21) suggest that impariment to diffusion may be caused by thromboses in the alveolar capillaries. They believe, however, that arterial unsaturation in polycythemia vera may also be caused by other pulmonary changes. They present evidence of impaired lung ventilation which they attribute both to damage to the respiratory centers and to a decrease in the elasticity of the lung tissue and an increase in its viscous resistance to changes in shape due to the increased viscosity of the blood. Such changes, they believe, may lead to arterial unsaturation by perfusion of poorly ventilated lung areas.

Bansi and Groscurth (23) found a displacement of the oxyhemoglobin dissociation curve to the left in the blood of one of three patients with polycythemia vera. They interpreted this displacement as a compensation to maintain the oxygen tension in the tissues at a normal level in the presence of increased hemoglobin in the blood. It is difficult to reconcile their findings with the results shown in Figure 1. The possibility of a pH effect should be considered. Bansi and Groscurth made no correction to a constant pH, while in the dissociation curves reported here the results of all equilibrations were corrected to a pH_e of 7.1 by methods described by Keys, Hall and Barron (7). Richards and Strauss (24) in a study of the oxygen dissociation curve in anemia included one case of polycythemia vera. The dissociation curve was normal.

In an earlier report (1) evidence was presented that in the polycythemia secondary to congenital heart disease the oxygen dissociation curve of the blood tends to be displaced to the right, this displacement being more pronounced in patients in whom blood flow to the lungs was reduced by congenital pulmonary stenosis. The essentially normal oxyhemoglobin curve of the blood of patients with polycythemia vera shows that the displacement of the curve in cyanotic congenital heart disease is not caused by polycythemia itself. The results seem to show that while the hemoglobin in the blood of polycythemia vera is normal in respect to its oxygen binding and dissociation characteristics the hemoglobin in the blood of polycythemic patients with cyanotic congenital heart disease is abnormal in these respects. While the number of patients studied was inadequate to substantiate this finding, the results suggest that this difference may prove useful in differentiating these types of polycythemia. It is not yet clear in which category of oxygen dissociation curve the polycythemia secondary to pulmonary disease will fall.

SUMMARY AND CONCLUSIONS

The oxygen capacity, the oxygen saturation and the acid-base properties of arterial blood were

studied in 17 patients with polycythemia vera. The results have been compared with the normal and with patients with polycythemia secondary to congenital heart disease. The arterial blood of five patients with polycythemia vera was studied before and after reduction of polycythemia by therapy. The effect of breathing pure oxygen upon the arterial saturation was determined in nine cases of polycythemia vera, and the oxygen dissociation curve was determined in eight patients. The results of these studies show:

1. Seven of the 17 patients in the study were found to have arterial saturations below the lower limit of normal, and in three patients the saturation was unquestionably low.

2. The finding of normal arterial saturation in some patients with polycythemia vera, including those with the highest hemoglobin, leads to the conclusion that polycythemia itself is not the cause of the arterial unsaturation. This conclusion is also supported by the variable effects of therapy upon the arterial saturation.

3. The observation that breathing oxygen raises the oxygen content of arterial blood to a degree comparable to that observed in normal arterial blood eliminated venous admixture as a cause of arterial unsaturation in polycythemia vera and suggests a pulmonary origin of unsaturation although not necessarily of polycythemia.

4. The oxygen dissociation of the blood was found to be normal. This is in contrast to a shift of the dissociation curve to the right in polycythemia secondary to congenital heart disease and indicates that the latter effect is not due to polycythemia alone. The results suggest that this difference may prove to be an important diagnostic aid.

5. Except for a decrease of the carbon dioxide combining power of the blood due to the higher concentration of the red cells in polycythemia vera, other acid-base properties of the blood in this disease, including the carbon dioxide combining power of the plasma, usually resemble the normal. A few isolated exceptions do not appear to be characteristic of the disease.

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URINARY FORMALDEHYDOGENIC CORTICOID AS DETERMINED AFTER ENZYMATIC HYDROLYSIS IN NORMAL SUBJECTS AND IN PATIENTS WITH ADRENAL AND HYPERTENSIVE DISEASE

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Corticosteroids appear in urine in free form and as conjugates. Most methods of assay of urinary corticoids involve acidification to pH 1 prior to extraction (1); increased yields thus obtained may be attributable to hydrolysis of steroid sulfates (2) since glucuronides are resistant to hydrolysis at pH 1 and room temperature. Treatment of urine with glucuronidase causes a further large increase in yield of both reducing (3) and formaldehydogenic (4, 5) corticoids because of hydrolysis of corticoid glucuronides. Measurement of urinary formaldehydogenic corticoids after enzymic hydrolysis should include all or nearly all the urinary content of C-21 steroids carrying α-ketol or α-glycol side chains.

Conditions of hydrolysis of urinary corticoid glucuronides have therefore been examined and procedures developed and applied to the assay of urines of normal subjects, of patients with essential hypertension, and of patients with diseases which affect the function of the adrenal glands. The two procedures used are similar in principle; they vary in detail and were developed and used independently; specimens were exchanged between the two laboratories and analyzed by both methods.

CONDITIONS OF HYDROLYSIS

Orienting experiments indicated that, using calf spleen β-glucuronidase (Viobin), maximal yields of formaldehydogenic corticoid (FC) were usually obtained after 48 hours' incubation at 37° C of a mixture of urine, buffer and enzyme to which chloroform was added as a preservative. Addition of penicillin and streptomycin did not enhance yields. In most of these experiments, the concentration of enzyme was 150 Fishman units per cubic centimeter of urine. The increments of FC with time did not exhibit the logarithmic progression characteristic of the action of the enzyme on a pure substrate (6), probably because of differences in rates of hydrolysis of the several corticoid glucuronide substrates which were ultimately measured together as FC.

The relatively slow release of FC in urine from glucuronide combination contrasts with the rapidity of the enzyme's action on pure steroid glucuronide substrates (7) and suggested to us that an enzyme inhibitor might be present in urine. After 1 hour, yields of phenolphthalein from incubation of phenolphthalein glucuronide, enzyme and urine were 40 to 70 per cent of those found in incubations of enzyme and substrate in water; the inhibition of the enzyme by the urine was roughly proportional to urinary specific gravity. It seemed that glucuronic acid, free or combined, might be the solute concerned, since the free acid is known to inhibit the action of β-glucuronidase (8), and noncorticoid glucuronides would compete for the enzyme. This assumption is supported by data which demonstrated the correlation between urinary inhibition of enzyme action and the concentration of free plus combined glucuronic acid in urine. However, the method used (9) does not discriminate between glucuronic acid and glucuronide.

Fortunately, the inhibition of enzyme action by urinary glucuronide or glucuronic acid does not interfere with complete hydrolysis of phenolphthalein glucuronide when the incubation is sufficiently prolonged.

The course of the hydrolysis can be speeded by increasing the temperature of incubation; maxi-

[1] This work was done during the tenure of a Post-Doctorate Research Fellowship, National Heart Institute, National Institutes of Health, United States Public Health Service.

mal yields of FC were obtained by incubation of urine, buffer and enzyme for 24 hours at 50° C (Procedure 2, below).

PROCEDURES

Principle. Hydrolysis of corticoid glucuronide with calf spleen β-glucuronidase, subsequent acidification and extraction of the mixture with chloroform yield what is assumed to be total FC (TFC); acidification and extraction of another appropriate aliquot permits determination of the FC fraction arbitrarily termed "free" (FFC), although it may include some FC derived from conjugates other than glucuronides; the difference between TFC and FFC in milligrams is the yield of corticoid derived from glucuronide (FCG). The values are calculated in terms of desoxycorticosterone.

Procedure 1

Reagents: 1. β-Glucuroniuase (Viobin), 25,000 Fishman units per gram.
2. Acetate buffer 1.0 M, pH 4.5 (6).
3. Chloroform C.P., freshly redistilled over potassium carbonate.
4. Other reagents as described for determination of FFC (10).

Apparatus: 1. Detergent-free, 125 cc. Erlenmeyer flasks.
2. Incubator, 37° C.
3. Automatic flask shaker (Burrell and Co., Pittsburgh).
4. Pipet and distillation apparatus (11).

Procedure. A 25 or 50 cc. aliquot of a 12 or 24 hour urine specimen is selected, the choice depending on the volume of the specimen, and transferred to a 125 cc. flask. The pH is brought to about 4.5 with HCl and secured at this level by addition of 0.1 volume of acetate buffer. Chloroform (10 cc.) is added, and 300 mg. (7,500 U.) of enzyme preparation admixed; the flask is closed with a foil-covered stopper and incubated at 37° C for 72 hours. The mixture is then brought to pH 1 with HCl and at once extracted with chloroform. Extraction and further procedures are as described by Corcoran and Page (10) appropriately adjusted for the smaller quantities of urine extracted.

Blank determinations are run in parallel with the tests on reagents, including enzyme. The concentration of FC is estimated from the difference in color density (D) of test and blank samples. Large inaccuracies may arise from this subtraction and multiplication of the result when color density of the test sample is low and urine volume large. When D as measured in a Coleman spectrophotometer, model 6A, is less than 0.1, the determination is usually repeated on a larger aliquot of urine; only exceptionally, as in cases of Addison's disease, have D values of 0.05 or less been accepted. We have taken a D of 0.025 as representing the possible maximal accumulation of errors in instrumentation and procedure. This color density corresponds to 0.018 mg. of desoxycorticosterone in the sample. Values reported hereinafter have been evaluated in terms of this potential error.

Procedure 2

Reagents: 1. Chloroform and β-glucuronidase (see Procedure 1).
2. Absolute alcohol. Commercial absolute alcohol distilled from 2,4 dinitrophenylhydrazine.
3. Periodic acid, 0.05 M in 0.3 N H_2SO_4.
4. Stannous chloride, 1.5 g. $SnCl_2 \cdot 2H_2O$ in 25 cc. of water containing 4 or 5 drops of concentrated hydrochloric acid. Prepare fresh daily.
5. Chromotropic acid. One gram recrystallized [2] sodium 1,8-dihydroxy-3,6-disulfonate is dissolved in 5 cc. of water in a 500 cc. Pyrex volumetric flask, 416 cc. of concentrated sulfuric acid is added cautiously with cooling, and the solution finally is diluted to volume with water. When stored in a brown glass-stoppered bottle the reagent prepared from the recrystallized salt will keep for two weeks without an appreciable increase in the blank.

Procedure. An aliquot of 1 to 2 per cent of the urine collected during a 24 hour period, but not more than 25 cc., is placed in a 50 cc. beaker. The aliquot is diluted to 25 cc. if necessary, and with the aid of a glass electrode pH meter the pH is adjusted to 4.8 with a 10 per cent solution of acetic acid. After addition of 6,000 units (240 mg.) of β-glucuronidase (3 cc. of a solution of the Viobin preparation, 2,000 units per cc. in 0.45 per cent sodium chloride) and 10 cc. of chloroform, the beaker is covered with a watch glass and placed in an incubator at 50° C. At the end of 24 hours, the mixture is diluted with 50 cc. of water and the aqueous phase is extracted by hand shaking in a separatory funnel four times with 20 cc. portions of chloroform. The chloroform extract is washed with a 5 per cent solution of sodium carbonate and then with water until neutral. Each aqueous extract is backwashed with a little chloroform which is added to the main extract. The chloroform is removed in an all-glass vacuum still, keeping the temperature of the water bath below 50° C. The residue is dissolved in 0.5 cc. of purified absolute

[2] Dissolve 10 g. practical grade sodium 1,8-dihydroxynaphthalene-3,6-disulfonate (Eastman No. P230) in 25 cc. of water by warming. Add about 0.2 g. Na_2SO_3, 5 to 10 drops of concentrated HCl and 1 g. activated charcoal. Heat on the steam bath 10 to 15 minutes. Filter hot with suction through a pad of infusorial earth and wash the filter with a little hot water. Warm the filtrate on the steam bath while slowly adding with stirring 200 cc. of acetone. If the acetone is added too rapidly at first the substance may separate as an oil. Once crystallization has started the acetone may be added more rapidly. Cool, filter, and dry the product in a vacuum desiccator over sulfuric acid.

TABLE I

*Urinary formaldehydogenic corticoids in normal subjects**

a. Procedure 1

	Males			Females			Both sexes	
	No.	Mean	Range	No.	Mean	Range	No.	Mean
FFC, mg.	17	1.2	0.34– 2.5	11	1.22	0.43– 2.1	28	1.22
TFC, mg.	20	24	7.1 –62	15	16.6	4.9 –33	35	21
FCG/FFC	17	19	8.0 –41	11	14	5.0 –27	28	17

b. Procedure 2

FFC, mg.	15	0.66	0.40– 0.86	8	0.66	0.46– 1.06	23	0.66
TFC, mg.	30	16.2	5.5 –39.0	18	13.5	6.8 –32.4	48	15.1
FCG/FFC	15	27.2	9.7 –52.7	8	28.5	12.3 –71.4	23	27.7

* Values obtained by each procedure in normal subjects. FFC is "free" formaldehydogenic corticoids in milligrams per 24 hours; TFC is urinary formaldehydogenic corticoid output as determined after enzymatic hydrolysis; FCG is the portion of TFC taken to be present as glucuronide; the ratio FCG/FFC demonstrates the increment in urinary corticoid yield obtained by enzymatic hydrolysis.

alcohol and 4.5 cc. of water is added. To a 2 cc. aliquot in a 50 cc. round-bottom flask with standard taper joint are added 2 cc. 5 N sulfuric acid and 1.0 cc. periodic acid. After 60 minutes at room temperature, excess periodic acid is destroyed with 1.0 cc. of stannous chloride, the formaldehyde is distilled and color is developed essentially according to the procedure of Corcoran and Page (10). An unoxidized blank is prepared by addition of 2 cc. 5 N sulfuric acid and 1 cc. of stannous chloride to another aliquot of the extract, then 1 cc. of periodic acid, followed by distillation. The distillate (approximately 5 cc.) is collected in a 10 cc. volumetric flask and diluted to 10 cc. Aliquots of 2 cc. are transferred to glass-stoppered test tubes and 5 cc. of chromotropic acid reagent is added. The color is developed by heating 0.5 hr. at 100° C.

The same procedure was used for determination of FFC except that the urine was acidified to pH 1, allowed to stand overnight at room temperature, and then extracted with chloroform.

Procedure 2 differs from Procedure 1 considerably in some respects. The enzyme is dissolved in 0.45 per cent sodium chloride solution. No extra buffer is added, but the pH has been checked repeatedly after incubation and found to be unchanged. Hydrolysis is accelerated by incubation at 50° C., and the enzyme remains active at this temperature. Under these conditions, the enzyme shows maximal activity at pH 4.8. From 82 to 105 per cent of cortisone added to the urine before incubation has been recovered. The average recovery in 16 experiments was 91 per cent. The incubation mixture is extracted at pH 4.8. This procedure may result in loss of the small amount of extra FC that ordinarily can be extracted at pH 1. However, the amount is negligible in comparison with the large amounts of FC liberated by β-glucuronidase. Cleaner extracts and more consistent results were obtained by extracting at pH 4.8.

TABLE II

Urinary formaldehydogenic corticoids of patients having Cushing's syndrome

Patient	Sex	Age, years	FFC,* mg. per 24 hours	TFC,* mg. per 24 hours	Remarks
1	F	15	1.3	17.9	Tumor
2	F	25	3.4	27.8	Tumor
3	F	27	1.1	14.6	Hyperplasia
4	F	34	2.5	9.0	Hyperplasia
5	F	37	1.7	13.1	Hyperplasia
6	F	21		45.0†	Carcinoma
7	M	30	2.0	24.4	Hyperplasia
8	M	31	1.4	23.4	Hyperplasia
9	M	40	2.0	45.0	Hyperplasia
10	M	50		21.4†	Hyperplasia
11	M	38	15.3	140.0†‡	Hyperplasia

* FFC, "free" formaldehydogenic corticoids. TFC, corticoids after enzymic hydrolysis.
† Procedure 1.
‡ Average of 3 determinations.

RESULTS

Normal subjects. Procedure 1—Analyses of 12 hour night, 24 or 48 hour urine specimens from laboratory and staff personnel carrying on their usual activities are summarized in Table I, a. Values of FFC in this series correspond generally with those previously reported (12); the difference previously observed as between males and females is not confirmed, possibly because of the smaller group examined. TFC outputs average 15 to 20 times those of FFC; these outputs are greater in males than in females. The proportion of corticoid excreted as glucuronide (FCG) to that excreted as FFC is widely variable.

Procedure 2—The normal subjects were similar to those described under Procedure 1. It will be noted that values (Table I, b) for FFC are distinctly lower than those obtained by Procedure 1, and the range of values is smaller. The reason

for these differences is not apparent. Probably because of the smaller values for FFC, the average ratio FCG/FFC calculated from the results of Procedure 2 is greater than that derived from the results of Procedure 1, although the average values of TFC and FCG are somewhat less. In general, results with Procedure 2 are of the same order of magnitude as those of Procedure 1.

Adrenal diseases. Cushing's syndrome, whether associated with hyperplasia or tumor of the adrenal cortex, is now considered to be a manifestation of overproduction of adrenocortical hormones. It would be expected, therefore, that an increased excretion of urinary corticoids would accompany this syndrome; indeed, an increase of FFC above the normal level is observed often. In those cases without a significant increase in FFC it was hoped that TFC would be increased much above the normal level, thereby facilitating the diagnosis of cortical hyperfunction. The results given in Table II show that, in general, this hope was not realized. The values for FFC were elevated definitely in 6 of the 9 cases for which data are available (cases 2, 4, 5, 7, 9, and 11); TFC could be considered to be definitely increased above the normal level only in cases 6, 9, and 11. However, the mean value of TFC (21.9 mg.) in the 8 cases in which procedure 2 was used, was increased above the mean of the normal values (15.1 mg.) determined by this procedure. Thus, it appears that FFC more often than TFC is indicative of adrenocortical hyperfunction associated with Cushing's syndrome.

Our data on untreated Addison's disease are few. In 3 such cases the values for FFC were 0.26, 0.32 and undetermined, and for TFC 2.2, 2.8 (Procedure 2) and 1.6 mg. (Procedure 1) per 24 hours, respectively. In one other case in which the disease evidently was not severe since the patient had maintained normal activities and had passed through two pregnancies uneventfully after the onset of pigmentation, the output of FFC was 0.28 and of TFC 22.0 mg. per 24 hours.

Essential hypertension. Nearly all these specimens were obtained from patients hospitalized in the ward of the Research Division, Cleveland Clinical Hospital, because of severe essential or, in some cases, malignant hypertension. The 80 determinations (Procedure 1) summarized in Table III are from 24 hour urine samples of 35 patients, of whom 16 were female; only 5 of the 80 determinations were done on specimens from patients whose renal failure had advanced sufficiently to cause azotemia.

The levels of FFC, in accord with former observations (12) are often increased and widely variable. The view was then expressed that this abnormality might be due to disproportionate outputs of FFC, rather than to increment in total corticoid. That this is the case is confirmed by the finding that outputs of TFC are not increased; thus, the increased outputs of FFC sometimes observed are associated with decreases in the ratio FCG/FFC.

Reproducibility of analyses. Duplicate samples analyzed by procedure 1 were found to agree within ± 15 per cent and usually within ± 10 per cent of the mean of the values obtained. Agreements in cortisone recoveries by Procedure 2 as noted earlier in this paper were within about 10 per cent of the mean. While each procedure thus seemed relatively satisfactory, it seemed desirable to test reproducibility severely by an exchange of samples between laboratories.

Results of this comparison are listed in Table IV. Among 23 specimens exchanged, 8 agree within 15 per cent and 14 within 24 per cent. The wide percentile variability between some of the remaining samples does not seem to be systematic, since the distribution is random. Making allowance for inherent methodological error equivalent to a color density of 0.025 on the galvanometer

TABLE III

*Urinary formaldehydogenic corticoids in hypertensive patients**

	Males			Females			Both sexes	
	No.	Mean	Range	No.	Mean	Range	No.	Mean
FFC, mg.	11	1.67	0.55–5.0	20	2.2	0.54–7.5	31	2.0
TFC, mg.	49	15.7	3.3–41	31	15.3	3.3–39.0	80	15.5
FCG/FFC	11	17	2.9–47	20	13	1.5–47	31	14.0

* Analyses by procedure 1. Terms as in Table I.

TABLE IV

*Comparisons of analyses by each procedure**

Patient	Procedure 1				Procedure 2				
	1	2	M	E	1	2	3	M	E
1	6.7			0.7	6.4	5.8		6.1	
2	7.8	7.3	7.5	2.2	5.8	6.0	6.2	6.0	
3	7.1			1.2	6.3	7.0	6.7	6.7	
4	29			1.5	27.0				4.5
5	28			1.7	24.4				3.7
6	25.4			1.2	21.3				3.7
7	15			1.0	13.1				2.9
8	16.8	14.2	15.5	0.9	15.3	16.7		16.0	1.8
9	21			1.2	21.6	19.5		20.6	1.8
10	18.2			1.0	19.4	21.0		20.2	1.6
11	14.8			1.3	12.7	12.8		12.8	2.0
12	1.7			0.9	2.2				
13	8.3	8.6	8.4	1.7	10.8	9.1	12.4	10.8	2.7
14	16.5			1.0	11.7				
15	24.4			0.7	16.3				1.3
16	5.5			0.5	2.8				
17	47.4			1.2	27.8				1.7
18	8.2			1.0	14.8				3.1
19	11			1.6	12.7	14.7		13.7	2.3
20	34			1.7	25.2	25.2		25.2	1.7
21	21.1			1.7	29.4	27.2		28.3	1.6
22	8.4			1.3	21.0				4.1
23	27.3	26.4	26.8	1.8	16.4	13.5	18.4	16.1	2.0

* Results of independent analyses of urine for TFC by each procedure expressed in milligrams per 24 hours. Numerical headings indicate the number of replicates analyzed, and M indicates the mean of these or the result of a single estimate. E is the potential error of each analysis as defined in the text and expressed in milligrams per 24 hours.

scale, 14 of 21 samples (excluding Nos. 14 and 16) agree completely. The desirability of duplicate rather than single analyses is suggested by the fact that in 4 instances of wide variation, single analyses were done by each procedure and in 2, by Procedure 1, with duplicates available by Procedure 2. In 2 instances (samples 14 and 16) in which the potential errors of Procedure 2 were not calculated, the absolute differences in TFC yields are relatively small—respectively, 4.8 and 2.7 mg. per 24 hours; single analyses only were available from each laboratory. The disparities in the cases of samples 17, 22, and 23 may be systematic; this interpretation is suggested in the case of sample 23 by the close intrinsic agreement between duplicates by Procedure 1 and triplicates by Procedure 2 and the disparate mean values obtained.

COMMENT

FC increment. The procedures confirm the presence in urine of amounts of TFC greatly in excess of those of FFC. Yields of reducing corticoids (RC) are similarly augmented by either enzymatic (3) or acid (13) hydrolysis; enzymatic yields of RC increase only "3 to 5 times" and with acid "4 to 5 times"; enzymatic hydrolysis in our hands increased yields of FC about 17-fold (range 5 to 70) and, in the hands of Cox and Marrian (5) "about 10 times." Thus, it seems that increments of FC on hydrolysis are greater than those of RC; the discrepancy can be explained if it is assumed that much of the urinary corticoid glucuronide is excreted with an α-glycol rather than an α-ketol side chain.

The chemical properties of the FC fraction of urine correspond to those of adrenal corticosteroids or metabolites thereof; it may be provisionally assumed that the bulk of this material is of adrenal cortical origin and that the amounts of TFC found in urine represent some fraction of the hormonal output of the adrenal gland. Actually, the mean level of TFC output in the urine of normal subjects corresponds roughly to the minimal maintenance requirement of cortisone in adrenalectomized human beings (14); the lability of TFC output in normal subjects exposed to the stresses and strains of daily life agrees with the known labile responsiveness of the pituitary-adrenal axis.

An extra-adrenal origin of urinary TFC has been suggested from observations on two Addisonian patients by Daughaday, Farr and Houghton (15), since in both cases the outputs of TFC after bacterial enzyme hydrolysis were within the normal range. The values for TFC in 2 other cases of Addison's disease were 1.7 and 0 mg. We have mentioned one patient with Addison's disease who excreted 22 mg. of TFC. Ward, Parkin, and Howell (16) have reported a case of co-existing Addison's disease and diabetes mellitus in which the FFC excretion was 0.66 to 1.06 mg. per 24 hours. The Addison's disease had developed some years after the diagnosis of diabetes. Although patients with this combination of diseases usually are very sensitive to insulin, the requirement for insulin in this case had not changed after the development of Addison's disease. This latter circumstance indicates that the patient was supplied with a fairly normal amount of carbohydrate-active adrenal hormones in spite of clinical Addison's disease. The normal values for FFC are in accord with this presumption. These observations suggest that Addison's disease is not always associated with complete failure of adrenocortical function. On this assumption, it is not necessary to conclude that the normal amounts of urinary formaldehydogenic material excreted by these unusual patients must be of extra-adrenal origin.

It is possible that some formaldehydogenic material may be of extra-adrenal origin since the value of FFC seldom is zero even in cases of long-standing severe Addison's disease or panhypopituitarism with clinical evidence of adrenal failure. More disturbing is the finding of Wilson (17) that most of the formaldehydogenic material freed by β-glucuronidase could not be recovered in the $C_{21}O_3$, $C_{21}O_4$, and $C_{21}O_5$ steroids separated by paper chromatography whereas these fractions of FFC gave titers comparable to the material applied to the paper. These results imply that the formaldehydogenic material extracted at pH 5 and also at pH 1 is steroid in nature but that most of that freed by enzymic hydrolysis is not steroid in nature. If that is true, then there would be no reason to expect correlation of the amount of this fraction with adrenal function. On the other hand, Cohen (7) has summarized evidence which lends support to the belief that the formaldehydogenic substances released by glucuronidase hydrolysis are indeed corticosteroids. Also, the amount of tetrahydrocortisone isolated by Baggett, Glick and Kinsella (18) after enzymic hydrolysis accounts for a fair proportion of TFC. At the present time these conflicting bits of evidence cannot be reconciled. In our opinion, the weight of evidence favors the view that urinary TFC is related to the adrenal cortex.

Essential hypertension. Levels of TFC are not increased in essential hypertension; indeed, the mean output in male patients is lower than that found in the normotensive males by Procedure 1, possibly because most of the patients were under the uniform and restful conditions of a stay in the hospital. In contrast, levels of FFC were frequently increased in both male and female patients; this may result either from some defect in corticoid conjugation or from intrarenal or intraurinary hydrolysis of preformed corticoid glucuronide. The presence of β-glucuronidase in renal epithelium, the potentiation of its activity by serum albumin (19), and the association of hypertensive renal disease with albuminuria and desquamation of renal epithelium argue in favor of the latter possibility.

Adrenal cortical disease and dysfunction. Levels in 3 patients with untreated Addison's disease are consistent with the concept that TFC accounts for a large proportion of the daily hormonal output of the adrenal cortex. The levels found in Cushing's syndrome do not accord too well with this view, since they are only exceptionally increased above the normal range. However, it may be significant that the mean value of TFC in 8 cases (Procedure 2) was increased 45 per cent over the mean of the normal values. Exceptionally, in 1 of the cases of Cushing's syndrome studied by Procedure 1, there was hypercorticoiduria both free and combined. The paradox of manifest hypercorticoidism without hypercorticoiduria, like that of hypocorticoidism without hypocorticoiduria, may depend on the metabolism of hormonal steroids. Thus, it may be that the diseased mesenchymal tissues of most patients with Cushing's syndrome "utilize" and degrade disproportionately the side chain of the excess steroid.

TFC output and urine volume. Since a large proportion of urinary 17-ketosteroids, like corticoid, is excreted as a glucuronide (20, 21, 22) and since it has been suggested that this excretion varies in part as a function of urine volume, TFC outputs obtained by Procedure 1 were examined for this association. Some association may be demonstrable in urines from normal subjects ($r = +0.43$, standard error ± 0.17); in hypertensive males, the respective values were $+0.43$, ± 0.15 and in hypertensive females $+0.19$, ± 0.18. Thus, the degree of association is not great. It may be methodological and due to the multiplication of small positive errors in analyses or urines of large volume.

SUMMARY

1. Determinations of urinary formaldehydogenic corticoid (FC) by two somewhat different procedures aimed at estimating total FC by treatment of urine with a preparation of spleen β-glucuronidase yield similar estimates in two groups of normal human subjects. The mean by Procedure 1 is 21 mg. and by Procedure 2 it is 15 mg. per 24 hours and the increments in FC effected by the enzyme preparation vary from 5- to 70-fold.

2. Low total FC (TFC) outputs were observed in 3 patients with untreated Addison's disease; normal outputs observed in some patients may depend on persistence of partial, albeit inadequate, cortical function. By Procedure 2, TFC outputs in 7 of 8 patients with Cushing's syndrome did not exceed the normal range, although the mean of the group was greater than the normal mean. In 1 of this group and in 2 of 3 analyzed by Procedure 1, TFC outputs were abnormally high.

3. Since outputs of TFC are within the normal range in hospitalized patients with severe essential hypertension, the increases in "free" FC sometimes observed in these patients are attributable to decreases in the proportion of FC excreted as glucuronide.

4. Independent analyses by each procedure were commonly in good agreement; the disparities sometimes found established the desirability of duplicate analyses.

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RENAL FUNCTION, ELECTROLYTE EXCRETION AND BODY FLUIDS IN PATIENTS WITH CHRONIC RENAL INSUFFICIENCY BEFORE AND AFTER SODIUM DEPRIVATION [1, 2]

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L'azotémie par manque de sel is a descriptive phrase coined by Blum, Graber, and van Caulaert (1, 2) to describe the azotemia and hypochloremia which developed in certain diabetic patients maintained on low salt diets immediately after recovery from coma. When sodium chloride was administered to these patients the plasma chloride concentration increased and nitrogen retention diminished or cleared. Pre-existing renal disease, anuria, or acidosis *per se* were not contributory factors. Blum and his associates (2) concluded that the development of azotemia was a compensatory response to decreased molecular concentration and osmotic activity of the blood. This view has not been widely accepted because it is known (3) that all membranes in the body are freely permeable to urea. Subsequent work with man (4) and other animals (5) has revealed that severe salt depletion is usually associated with loss and redistribution of body water that may lead to circulatory collapse and renal dysfunction.

Marked sodium depletion is necessary to induce renal vasoconstriction and diminished glomerular filtration sufficient to cause nitrogen retention in normal human subjects and it is usually associated with obvious dehydration, weight loss, muscular weakness, apathy, and anorexia (4). In patients with renal insufficiency secondary to renal disease much less severe sodium depletion suffices to precipitate or to intensify nitrogen retention (6). Since renal disease often appears to predispose to easy loss of body water, dehydration may also be an important factor in this situation.

Wiggins, Manry, Lyons, and Pitts (7) have found that moderate salt depletion induces almost complete cessation of sodium excretion in normal man, chiefly as a result of increased tubular reabsorption. In the uremic patient it is possible that this mechanism is deranged and that changes in filtration are necessary to prevent undue loss of sodium in the urine. An investigation was therefore undertaken to define the relative importance of changes in the volume of body fluids, in the distribution of sodium and potassium in the body, and in renal function in the production of increased azotemia during moderate sodium deprivation in patients with chronic renal insufficiency

METHODS

Two subjects of these studies were normal men clinically free from cardiovascular or renal disease; eight subjects suffered from chronic renal insufficiency with nitrogen retention as a result of well-established chronic diffuse glomerulonephritis (four), malignant arteriolar nephrosclerosis (two), chronic pyelonephritis (one) and polycystic renal disease (one). All were free of edema or other evidences of heart failure. Arterial hypertension had developed in all and anemia in seven.

Deprivation without depletion of sodium was effected by placing five uremic subjects (G. B., M. B., C. P., J. F., and T. C.) on a low salt diet (18 to 25 mEq. of sodium per day and 1 Gm. protein per Kg. body weight) and sodium-exchanging resin [7] (60 gm. per day in divided doses) for a five day period. With one exception all were maintained on a regular hospital diet containing 70 to 105 mEq. of sodium per day for at least one week prior to sodium deprivation. In the exceptional instance (T. C.)

[1] These studies have been made possible through the aid of a grant from the New York Heart Association.

[2] Preliminary report of some of these data has been published (37).

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[7] We are indebted to Dr. K. G. Kohlstaedt and the Eli Lilly Co., Indianapolis, Indiana, for a generous supply of carboxylic acid exchange resins (Carbo-Resin).

a low sodium diet (23.6 mEq. sodium per day) had been provided for three days. The low salt diet was discontinued and supplemented by salt tablets (85 mEq. sodium per day) for five days following termination of resin intake in three patients (C. P., J. F., and T. C.) in order to examine the effect of salt repletion. In one uremic subject (T. C.) accurate measurements of the sodium, potassium and water balance were made while in residence on the Metabolism Service of the Presbyterian Hospital. One of the two normal subjects (A. B.) was also studied in the same manner during a five day period of more rigorous sodium withdrawal (18.5 mEq. sodium per day and 120 gm. of cation-exchange resin daily in divided doses) followed by sodium repletion as described above.

Measurements of inulin, *p*-aminohippurate (PAH),[8] sodium, potassium, chloride and phosphate clearances and determination of the maximal rate of tubular PAH secretion (Tm_{PAH}) (8, 9) were made just before and at the close of the periods of sodium deprivation and repletion. In addition, plasma carbon dioxide content (10), plasma calcium concentration (11), and blood urea nitrogen (12) were measured at these times. The urinary outputs of sodium and potassium were measured daily in all. Fecal losses of sodium and potassium were also determined in the two subjects on "balance study" on the Metabolism Service. Sodium and potassium concentrations in plasma and urine were determined with an internal standard flame photometer (13), chloride by the method of Schales and Schales (14), inorganic phosphate by the method of Fiske and Subbarow (15) and pH (Cambridge glass electrode) in urine samples collected under oil.

The day preceding or following clearance study measurements of the volume of distribution of antipyrine[9] (16), inulin (17), and T-1824 (18) were made in five patients with renal insufficiency. PAH was found to interfere markedly with antipyrine determinations and PAH clearances could not be measured simultaneously. Since inulin excretion was greatly reduced in all these individuals the difference between the amount of inulin excreted during equilibrium and the amount injected was large enough to measure accurately. The amount trapped in the renal "dead space" was relatively small and the method of Gaudino, Schwartz, and Levitt (17) could be safely modified as follows: *1*) exactly five grams of inulin in five per cent dextrose were administered by intravenous infusion over a two-hour period; *2*) urine was collected simultaneously by indwelling catheter during this period and for the three hours thereafter allowed for equilibration; *3*) the plasma inulin concentration (mg. per ml.) determined at this time (five hours after beginning the inulin infusion) was divided into the total amount remaining in the body (the amount infused less the amount excreted in the urine during the five hour period) to obtain values for the volume of distribution of inulin. In every instance, the plasma inulin concentration at "equilibrium" was higher than 20 mg. per cent. The "intracellular fluid volume" was estimated as the difference between the inulin and antipyrine "spaces." The hematocrit was determined using venous blood obtained without stasis.

"Total exchangeable" sodium and potassium were determined (19, 20) in three uremic subjects by isotope dilution (Na^{24}, 1.2 μc. per Kg. body weight; K^{42}, 1.5 μc. per Kg. body weight). The isotope was injected intravenously, urine carefully collected over a 24 hour period and the "exchangeable" mass computed from the plasma specific activity at the end of this period and the amount of isotope remaining in the body, allowing for decay and elimination in the urine. In three subjects the volume of distribution of Na^{24} four hours after injection (21) was determined for comparison with simultaneously determined inulin "space." The distribution of "total exchangeable" sodium and potassium between "cells" (and bone) and extracellular fluid was calculated on the basis of simultaneously determined inulin and antipyrine "spaces."

The body weight was measured daily and the blood pressure determined sphygmomanometrically twice daily throughout each study.

RESULTS

Electrolyte and water excretion during renal insufficiency. The data presented in Tables I to III, obtained in studies of eight patients with chronic renal insufficiency, show that sodium, potassium, chloride and water excretion were usually increased above normal, relative to glomerular filtration. The greater loss of filtered electrolyte in the urine (electrolyte-inulin clearance ratio, C_e/C_{in}, or the percentage of filtered electrolyte excreted) is indicative of diminished tubular reabsorption that serves to maintain electrolyte balance. Normally, little more than 25 per cent of the water, sodium and chloride in the glomerular filtrate appears in the urine even under heavy loads for excretion (22), whereas as much as 63 per cent of water, 18 per cent of sodium, and 17 per cent of chloride in the filtrate (Tables I and II) were excreted by patients in this series at rest. Potassium excretion fell into a special category because it actually exceeded the amount filtered in five of the eight subjects (Table I), in contrast to the normal where more than 20 per cent seldom appears in the urine (23) except under conditions of loading. This phenomenon has also been described by Keith (24), Earle (25), Platt (26) and their colleagues who interpret it as evidence for active tubular secretion of potassium. These

[8] We wish to thank Dr. W. Boger and the Medical Research Division of Sharp and Dohme, Inc., Philadelphia, Penna., for a generous supply of sodium *p*-aminohippurate.

[9] Supplied through the courtesy of Dr. K. G. Kohlstaedt and the Eli Lilly Co., Indianapolis, Indiana.

defects in renal electrolyte excretion were not associated with any serious disturbance in plasma electrolyte composition in these selected patients (Table III).

The maximal tubular PAH secretion was reduced to approximately the same extent as filtration, the inulin clearance-Tm_{pah} ratio (which normally averages 1.5 ml. per mg.) amounting to 1.76, 1.50, 0.70, 1.18, and 1.35 ml. filtrate per mg. of secreted PAH (Table III) in the five subjects in whom the measurement was made. A study of electrolyte excretion in three of these subjects during PAH revealed (Table II—control studies) little or no changes in pH, phosphate clearance (C_p/C_{in}) and urine flow (V/C_{in}) relative to the inulin clearance. In one normal subject (A. C.—control, Table II) studied under the same conditions, pH fell sharply and both C_p/C_{in} and V/C_{in} increased, as noted previously by West and Rapoport (*27*). The percentage of filtered sodium, potassium and chloride excreted in the urine (C_{na}/C_{in}, C_k/C_{in}, and C_{cl}/C_{in}) increased during PAH loading in both the normal and uremic subjects (with the exception of M. B., Table II—in whom C_k/C_{in} fell).

Effect of sodium deprivation on electrolyte ex-

TABLE I

Proportion of filtered water and electrolytes excreted in the urine of patients with chronic renal insufficiency

Sex	Patient, diagnosis, age	S.A.	BUN	C_{in}	C_{pah}	F.F.	P_{Na}	C_{Na}/C_{in}	P_k	C_k/C_{in}	V/C_{in}
		m.²	*mg %*	*ml./min.*	*ml./min.*	*%*	*mEq./L.*	*%*	*mEq./L.*	*%*	*%*
F	M. D. Chronic glom. neph. 30	1.72	84	2	13	15	128	6.0	3.1	512	38
M	T. A. Chronic glom. neph. 21	1.68	83	3	10	30	134	6.0	3.2	291	31
M	F. M. Chronic pyeloneph. 40	1.81	62	7	40	18	134	12.0	3.8	116	24
F	C. P. Polycystic disease 57	1.87	66	7	25	28	135	17.7	3.8	227	63
M	M. B. Malig. neph. scler. 28	1.91	77	12	50	24	136	12.7	3.4	119	23
M	J. F. Chronic glom. neph. 56	1.47	44	20	105	19	134	6.9	4.8	51	11
M	G. B. Malig. neph. scler. 21	1.86	32	30	100	30	132	1.3	3.6	53	4
M	T. C. Chronic glom. neph. 54	1.70	40	50	250	20	138	2.0	5.0	40	7

All values for renal clearances and urinary sodium and potassium excretion are averages of three successive determinations.

Abbreviations are as follows:

S.A. = Surface Area (M^2).
C_{in} = Inulin clearance.
C_{pah} = Sodium *p*-aminohippurate clearance.
FF = Filtration fraction (C_{in}/C_{pah}).
P_{Na} = Plasma sodium concentration.
C_{Na}/C_{in} = Sodium-inulin clearance ratio (percentage of filtered sodium excreted).
P_k = Plasma potassium concentration.
C_k/C_{in} = Potassium-inulin clearance ratio (percentage of filtered potassium excreted).
V/C_{in} = Urine flow-inulin clearance ratio (percentage of filtered water excreted; no correction for free water in the plasma has been made, since the error introduced is negligible).

cretion. Sodium deprivation by low salt diet and sodium-exchange resin resulted in a sharp decrease in sodium excretion ($U_{Na}V$-mg. per min., Table III) to 2.8 per cent of control in one normal subject (A. C.—control, Table III, preliminary control figures were not obtained in A. B.) and to 36.0, 72.5, 20.2, and 32.2 per cent of control in four of five uremic patients (Table III). There was no significant change in sodium output in C. P., a woman with long-standing renal insufficiency due to polycystic disease. Potassium output fell from 53.1 μEq. per min. to 10.4 μEq. per min. in the normal subject (A. C.—Table III) but showed no consistent change in direction or magnitude among the uremics, rising in C. P. from 58.6 to 98.3 μEq. per min. and in M. B. from 47.2 to 58.2 μEq. per min., falling in J. F. from 47.7 to 33.4 μEq. per min., and remaining relatively unchanged in the other patients (G. B. and T. C.).

The values for electrolyte excretion given in Tables I to III are those measured during determination of clearances and are not to be construed as averages or representative of the output during the course of each day. The methods of measurement, diurnal variation (28), and other factors might be expected to affect these figures, but since they were obtained under standard conditions it seems reasonable to attribute any difference between values before and after deprivation to the effect of deprivation alone. The average minute output of sodium during each day of deprivation was probably much less than the values

TABLE II

The effect of intravenous PAH loading on urine pH and urinary electrolyte excretion

Patient		Regimen	C_{in}	pH	C_{Na}/C_{in}	C_K/C_{in}	C_{Cl}/C_{in}	C_p/C_{in}	V/C_{in}
			ml./min.	*units*			%		
Normal Renal Status									
A. C.	Control	Before	105	6.9	1.45	14.80	1.25	8.55	1.63
		PAH load	70	5.4	4.16	17.90	1.57	17.55	3.20
	Na depriv.	Before	105	6.3	0.04	3.20	0.25	14.03	5.00
		PAH load	95	4.7	1.00	6.90	0.32	19.43	5.69
A. B.	Na depriv.	Before	120	6.5	0.01	0.93	0.55	7.10	1.38
		PAH load	80	4.7	0.39	10.71	0.72	19.14	3.73
	Repletion	Before	125	5.8	0.14	7.55	0.51	3.55	0.53
		PAH load	120	5.0	1.43	11.47	0.87	10.03	1.23
Chronic Renal Insufficiency									
M. B.	Control	Before	12	6.3	12.70	118.90	16.90	9.71	22.50
		PAH load	14	6.3	18.20	102.30	21.10	9.79	28.10
	Na depriv.	Before	7	6.2	17.00	249.00	23.70	5.56	36.60
		PAH load	6	6.2	25.30	259.70	31.30	5.14	40.90
J. F.	Control	Before	20	5.7	6.90	50.80	9.10	11.50	10.70
		PAH load	19	5.7	21.20	62.30	12.00	13.80	12.20
	Na depriv.	Before	12	4.8	2.30	61.60	3.70	10.60	23.00
		PAH load	12	4.7	6.70	80.30	4.70	10.30	31.70
	Repletion	Before	26	5.7	7.90	51.20	9.70	15.30	13.50
		PAH load	23	5.7	14.70	56.30	14.50	14.20	22.60
T. C.	Control	Before	50	5.2	1.95	39.50	3.28	15.30	6.65
		PAH load	40	5.3	7.85	54.80	6.26	18.91	11.72
	Na depriv.	Before	30	4.4	0.85	67.00	1.42	21.53	7.80
		PAH load	30	4.6	5.16	102.50	2.72	19.27	10.07
	Repletion	Before	45	4.9	4.05	28.10	2.80	18.61	4.22
		PAH load	45	5.0	12.26	34.90	11.36	21.29	10.86

All values are averages of three determinations.
Abbreviations are as follows; otherwise as in Table I:

Regimen: *Control*, on diet containing 70 to 105 mEq. sodium daily, at least 1 gm. protein per Kg. daily, and adequate caloric provision;
Na depriv., sodium intake reduced to 18 to 25 mEq. daily, and cation exchange resin (Carbo-resin) 60 gm. daily in divided doses;
Repletion, control diet and supplement of 85 mEq. sodium daily. Each study at termination of a period of at least five days under each regimen.

C_{Cl}/C_{in}: Chloride-inulin clearance ratio (percentage of filtered chloride excreted).
C_p/C_{in}: Phosphate-inulin clearance ratio (percentage of filtered phosphate excreted).

TABLE III

The effect of sodium deprivation on body weight, renal function, urinary sodium and potassium excretion, and on plasma electrolytes

Sex	Patient Diagnosis Age	S.A.	Regimen*	Body wt.	C_{in}	C_{pah}	F.F.	Tm_{pah}	$U_{Na}V$	U_kV	Na	K	CO_2	Plasma Cl	Ca	P	BUN
		$m.^2$		*Kg.*	*ml./min.*	*ml./min.*	%	*mg./min.*	*μEq./min.*	*μEq./min.*	*mEq./L.*				*mg. %*		
Normal Renal Status																	
	A. C.																
	Rheum. arthritis		Control	43.6	105	755	14	65	203.6	53.1	137	3.5	24.2	99.7	9.4	4.1	10
F	37	1.49	Na depriv.	42.8	105	550	19	35	5.8	10.4	134	3.1	22.9	99.4	10.0	4.2	11
	A. B.																
	Pulm. emphysema		Na depriv.	64.7	120	505	24	40	1.2	3.7	137	4.2	28.8	96.8	9.7	3.1	15
M	45	1.70	Repletion	66.2	125	575	23	50	27.3	48.9	140	5.0	26.4	103.0	9.4	3.3	13
Chronic Renal Insufficiency																	
	G. B.																
	Malig. neph. scl.		Control	62.6	30	100	30	17	48.7	53.0	132	3.6	29.3	94.8	9.5	5.1	32
M	21	1.86	Na depriv.	62.5	15	60	25	8	17.5	51.3	132	3.0	28.0	93.0	9.1	5.4	30
	M. B.																
	Malig. neph. scl.		Control	68.5	12	50	24	8	205.3	47.2	136	3.4	22.6	96.4	9.1	9.4	77
M	28	1.91	Na depriv.	67.5	7	25	28	4	149.3	58.2	132	3.4	23.0	91.2	8.7	10.4	102
	C. P.		Control	82.0	7	25	28	10	165.6	58.6	135	3.8	22.0	103.5	6.9	6.9	66
	Polycystic disease		Na depriv.	81.2	8	15	48	4	162.3	98.3	136	3.5	24.9	99.8	7.6	7.8	64
F	57	1.87	Repletion	84.0	12	25	45	4	319.5	91.3	142	3.4	23.5	104.5	7.3	7.0	60
	J. F.		Control	50.2	20	105	19	17	166.9	47.7	134	4.8	21.6	109.3	9.1	4.3	44
	Chronic glom. neph.		Na depriv.	48.6	12	95	12	13	33.7	33.4	131	4.7	19.1	107.0	8.5	5.5	63
M	56	1.47	Repletion	51.4	26	125	21	13	277.0	67.5	136	5.0	23.0	108.2	9.6	4.1	37
	T. C.		Control	61.6	50	250	20	37	129.0	100.3	138	5.0	22.0	103.0	9.7	4.0	40
	Chronic glom. neph.		Na depriv.	60.8	30	230	14	39	41.6	98.5	137	4.1	19.9	106.7	10.1	5.4	65
M	54	1.70	Repletion	60.9	45	295	16	38	264.2	59.7	140	4.7	21.9	104.1	10.3	4.4	38

* Regimens as noted under Table II.

All values for renal clearances and urinary sodium and potassium excretion are averages of three determinations.

Abbreviations are as follows; otherwise as in Tables I and II:

Tm_{pah} = maximal tubular excretion of PAH.
$U_{Na}V$ = urinary sodium output.
U_kV = urinary potassium output.

for $U_{na}V$ presented in Table III. Thus, careful measurement of sodium balance in one uremic patient (T. C.—Table IV) revealed that urinary sodium excretion during deprivation ranged from 4.5 to 7.4 mEq. per day, or from 3.1 to 5.1 μEq. per min. on the average, as compared with the value of 41.6 μEq. per min. observed during the clearance study.

The resin, in the doses used, assured a loss of sodium in the stool approximately equal, in T. C. (Table IV), to intake, resulting in a slightly negative sodium balance that totalled 28.8 mEq. for the five days of deprivation. This small deficit was insufficient to produce any obvious change in plasma sodium concentration (Table III). The resin provided enough potassium to permit a small potassium gain of 15.8 mEq. over the five days of administration in T. C. (Table IV) without any effect on plasma potassium concentration. It may be presumed that very small changes in electrolyte balance occurred similarly in the other subjects on the same regimen. This assumption finds support in the absence of much change in the plasma electrolyte pattern (Table III). However, the plasma phosphate concentration (P, Table III) did tend to rise and the blood urea nitrogen increased decidedly in three of the five uremic patients, by 35, 43, and 62 per cent (Table III).

Effect of sodium deprivation on glomerular filtration and tubular function. The increment in nitrogen retention was approximately proportional to the decrement in inulin clearance. Only in G. B. (Table III) was there a decrease in the inulin clearance without alteration in blood urea

nitrogen concentration. It seems unlikely that any defect in the inulin clearance as a measure of filtration under these conditions can account for a change of the magnitude observed (to 50, 58, 60, and 60 per cent of control—Table III) and these figures may be accepted, therefore, as evidence for diminished filtration. PAH clearance also decreased (Table III) and it may be presumed that intrarenal vasoconstriction lowered renal blood flow and filtration pressure, thus accounting for the fall in filtration rate. PAH clearance decreased by 27 per cent in the normal subject (A. C.—Table III) on the same regimen, without change in the inulin clearance. The renal extraction is always impaired in renal insufficiency (9) and it is possible that the decrement in PAH clearance was attributable, at least in part, to further reduction in extraction. Measurements of renal extraction of PAH before and after sodium deprivation are necessary to clarify this point.

The fall in filtration accounted not only for retention of nitrogen but it was also responsible for the fall in sodium excretion in patients with renal insufficiency. In the normal subject (A. C. Table III) increased tubular reabsorption alone appeared to be sufficient. To test this point, a normal subject (A. B.) in whom electrolyte and water balance were carefully controlled was placed on a more rigorous regimen of dietary salt restriction (18.5 mEq. per day) and resin administration (120 gm. per day in divided doses) (Table V). Within a period of five days there was a net loss of 214 mEq. of potassium and 529 mEq. of sodium (or almost twenty times that observed in the uremic patient [T. C.] under the usual regimen). The glomerular filtration rate following this period was 120 ml. per min., a value well within normal limits, and there was no evidence of phosphate or nitrogen retention. These results agree with those of Wiggins and his associates (7) who found that the glomerular filtration rate did not change significantly and that nitrogen retention did not occur in 17 normal subjects receiving a low salt diet and 2 ml. of a mercurial diuretic (Mercuzanthin) daily, a regimen sufficient to produce significant reduction in plasma sodium concentration and in body weight during a three-day period.

Sodium output fell proportionately more than

TABLE IV

Sodium, potassium, and water balance during sodium deprivation and repletion in chronic uremia

T. C.	Uremia		Sodium *mEq./24 hr.*				Potassium *mEq./24 hr.*				Fluid *ml./24 hr.*	
				Excretion				Excretion				
Period	Hosp. day	Body wt.	Intake	Urine	Stool	Balance	Intake	Urine	Stool	Balance	Intake	Urine volume
		Kg.										
I	4	61.6	63.0*	31.9	8.5	+ 22.6	92.4	68.6	18.3	+ 5.5	1,930	1,625
Control‡	5	61.9	23.6	11.6	8.5	+ 3.5	92.4	72.6	18.3	+ 1.5	1,930	1,680
			Net balance			+ 26.1	Net balance			+ 7.0		
			Total exch. body Na = 2,475 mEq.				Total exch. body K = 2,355 mEq.					
	6	61.5	23.6	6.3	23.2	− 5.8	182.4†	76.4	111.0	− 5.0	1,930	1,330
	7	61.5	23.6	7.4	23.2	− 7.0	182.4	62.0	111.0	+ 9.4	1,930	1,405
II	8	61.2	23.6	6.7	23.2	− 6.3	182.4	80.0	111.0	− 8.6	1,930	1,530
Na depriv.	9	61.1	23.6	4.5	23.2	− 4.1	182.4	63.2	111.0	+ 8.2	1,930	1,200
	10	60.8	23.6	6.0	23.2	− 5.6	182.4	59.6	111.0	+11.8	1,930	1,460
			Net balance			− 28.8	Net balance			+15.8		
			Total exch. body Na = 2,440 mEq.				Total exch. body K = 2,410 mEq.					
III	11	60.5	61.9*	6.0	6.1	+ 49.8	102.5	50.2	61.2	− 8.9	1,930	1,520
Control	12	61.1	20.5	7.4	6.1	+ 7.0	102.5	41.5	61.2	− 0.2	1,930	1,460
			Net balance			+ 56.8	Net balance			− 9.1		
	13	60.7	174.5	13.5	12.6	+148.4	102.5	52.5	16.1	+34.2	1,930	1,410
	14	60.5	174.5	53.9	12.6	+108.0	102.5	69.2	16.1	+17.2	1,930	1,420
IV	15	60.9	225.5	140.8	12.6	+ 72.1	102.5	71.2	16.1	+15.2	1,930	1,675
Repletion	16	60.7	225.5	190.0	12.6	+ 22.9	102.5	71.4	16.1	+15.0	1,930	1,710
	17	60.9	225.5	212.2	12.6	+ 0.7	102.5	65.9	16.1	+20.5	1,930	1,710
			Net balance			+352.1	Net balance			+102.1		
			Total exch. body Na = 2,885 mEq.				Total exch. body K = 2,535 mEq.					

* Denotes additional sodium administered as sodium *p*-aminohippurate in measuring Tm_{pah}.

† Denotes additional potassium administered in sodium removing resins (each 1 gm. resin contained 1 mEq. of potassium).

‡ Regimen in each period as noted under Table II.

TABLE V

Sodium, potassium, and water balance during sodium depletion and repletion in normal man

A. B. Period	Normal Hosp. day	Body wt.	Sodium mEq./24 hr. Intake	Excretion Urine	Stool	Balance	Potassium mEq./24 hr. Intake	Excretion Urine	Stool	Balance	Fluid ml./24 hr. Intake	Urine volume
		Kg.										
	10	66.7	Total exch. body Na = 2,730 mEq.									
	11	66.1	18.5	0.4	130.0	−111.9	257.3†	25.2	290.0	−57.9	1,900	1,810
	12	65.5	18.5	0.3	130.0	−111.8	257.3	18.8	290.0	−51.5	1,900	2,090
I Na deplet.‡	13	65.2	18.5	0.4	120.0	−101.9	257.3	14.2	281.4	−38.3	1,900	1,970
	14	65.2	18.5	0.2	120.0	−101.7	257.3	9.2	281.4	−33.3	1,900	1,840
	15	64.7	18.5	0.5	120.0	−102.0	257.3	9.3	281.4	−33.4	1,900	1,730
				Net balance =		−529.3		Net balance =		−214.4		
			Total exch. body Na = 2,132 mEq.									
II Control	16	64.5	18.5	0.4	51.5	− 33.4	77.3	5.0	149.2	−76.9	1,900	1,440
	17	64.3	61.3*	0.5	51.5	+ 9.3	77.3	3.9	149.2	−75.8	1,900	1,860
				Net balance =		− 24.1		Net balance =		−152.7		
	18	64.0	103.5	0.6	1.5	+101.4	77.3	3.1	12.3	+61.9	1,900	920
	19	64.6	103.5	0.8	1.5	+101.2	77.3	10.5	12.3	+54.5	1,900	900
III Repletion	20	65.6	103.5	1.9	1.5	+100.1	77.3	29.8	12.3	+35.2	1,900	810
	21	65.8	103.5	10.9	1.5	+ 91.1	77.3	54.0	12.3	+11.0	1,900	900
	22	66.2	103.5	19.8	1.5	+ 82.2	77.3	50.0	12.3	+15.0	1,900	750
				Net balance =		+476.0		Net balance =		+177.6		
			Total exch. body Na = 3,010 mEq.									

* Denotes additional sodium administered as sodium *p*-aminohippurate in measuring Tm_{pah}.
† Denotes additional potassium administered in sodium removing resins (each 1 gm. resin contained 1.5 mEq. of potassium).
‡ Na deplet.: sodium intake reduced to 18.5 mEq. per day and 120 gm. of a cation exchange resin (Carks-resin) given each day in divided doses. Regimen and abbreviations otherwise as under Table II.

filtration in G. B., J. F., and T. C. (Table III), indicating that augmented tubular reabsorption also may play a role in sodium conservation in uremia. In M. B. tubular reabsorption was greatly impaired and showed no evidence of increase during deprivation. Tubular dysfunction was also apparent in a fall in Tm_{pah} in 4 of the 5 uremics. There was no change in the lack of ability to acidify the urine during PAH loading (Table II).

Cessation of resin administration and the addi-

TABLE VI

Effect of sodium deprivation on the volume of the body fluid compartments in patients with chronic renal insufficiency

Patient	Regimen*	Body wt.	Total body water		Extracellular fluid		Intracellular water		Plasma volume		Blood volume		Hct.
		Kg.	L.	% body wt.	L.	% body wt.	L.	% body wt.	L.	% body wt.	L.	% body wt.	%
G. B.	Control	62.6	31.0	49.5	14.5	23.2	16.5	26.5	3.9	6.3	6.4	10.0	38
	Na depriv.	62.5	30.0	48.0	16.5	26.5	13.5	21.6	4.3	6.9	6.3	10.0	31
M. B.	Control	68.5	37.5	54.8	14.5	21.2	23.0	33.5	3.6	5.2	5.0	7.3	32
	Na depriv.	67.5	32.5	48.0	15.5	23.0	17.0	25.2	3.8	5.6	5.1	7.5	25
C. P.	Control	82.0	41.0	50.0	16.5	20.2	24.5	30.0	3.7	4.6	5.5	6.7	33
	Na depriv.	81.2	44.0	54.0	13.0	16.0	31.0	38.2	3.9	4.8	5.7	7.0	32
	Repletion	84.0	45.5	54.2	16.5	19.6	29.0	34.5	3.7	4.4	5.8	6.9	29
J. F.	Control	50.2	31.0	61.2	12.5	25.0	18.5	36.2	2.9	5.9	4.2	8.3	29
	Na depriv.	48.6	30.5	62.8	12.0	24.6	18.5	39.0	3.0	6.2	4.2	8.7	29
	Repletion	51.4	31.0	60.2	15.0	29.0	16.0	31.2	3.3	6.3	4.3	8.4	22
T. C.	Control	61.6	31.0	50.2	15.5	25.2	15.5	25.2	2.9	4.7	4.9	8.1	43
	Na depriv.	60.8	30.0	49.5	15.0	24.6	15.0	24.6	3.1	5.1	4.9	8.1	36
	Repletion	60.9	31.0	51.0	16.0	26.4	15.0	24.6	3.4	5.5	4.9	8.1	32

* Regimen as noted under Table II.

TABLE VII

Comparison of simultaneous measurements of the four-hour Na^{24} space and the inulin space in patients with chronic renal insufficiency

Patient	Regimen*	Body wt.	Na^{24} space		Inulin space	
				% body wt.		% body wt.
		kg	*L.*		*L.*	
C. P.	Control	82.0	22.0	27.0	16.5	20.0
T. C.	Control	61.6	18.8	30.5	15.5	25.2
	Na depriv.	60.8	18.0	29.6	14.9	24.5
	Repletion	60.9	19.5	32.0	16.1	26.4
J. F.	Repletion	51.4	18.4	35.8	14.9	29.0

* Regimen as noted under Table II.

tion of salt to the diet resulted in a tendency for all values (except Tm_{pah}) to return to or rise above the control levels in 3 uremic patients (C. P., J. F., and T. C.—Repletion, Table III), indicating the reversibility of the changes occurring during deprivation. The failure of Tm_{pah} to recover has also been noted by Chasis and his co-workers (29) in a study of the effect of salt restriction in patients with essential hypertension.

Body water and electrolyte distribution during renal insufficiency. Measurements of the volume of total body water (antipyrine "space") in five patients with renal insufficiency (Table VI) yielded values well within normal limits, ranging from 49.5 to 61.2 per cent of body weight. Since all were spare and non-edematous this finding was not unexpected. But the inulin "space" (extracellular water volume) was greater than normal in every instance, ranging from 20.2 to 25.2 per cent (in contrast to the normal range from 11.4 to 18.0 per cent of body weight [30]) and the volume of intracellular fluid was correspondingly reduced. Determination of the volume of distribution of radiosodium four hours after administration in three of these subjects also yielded higher than normal values (Table VII). As in normals, the four-hour radiosodium "space" (21) exceeded the inulin "space." The blood volumes (T-1824) were all within normal limits.

The "total exchangeable" sodium and potassium were within normal limits in three subjects, two of whom were studied during the control period (C. P. and T. C.—Table VIII) and one during sodium repletion only (J. F.—Table VIII). Calculation of the electrolyte concentrations within the "cells" (and bone), in these patients (using simultaneously determined antipyrine and inulin "spaces") revealed concentrations of 26, 22, and 28 mEq. sodium per liter of cell fluid (normal range — 31 to 44 mEq. per liter [31]) and 105, 150, and 120 mEq. potassium per liter (normal range — 96 to 125 mEq. per liter [31]) suggesting that loss of sodium from the cells and/or bone may have occurred during the course of renal insufficiency despite maintenance of extracellular fluid concentrations. However, the validity of the methods available for the estimation of extracellular fluid volume is open to serious question in uremia where changes in cellular permeability could permit rapid escape of inulin or radioactive sodium into the cells and this conclusion cannot be accepted without reserve.

Effect of sodium deprivation on body water and electrolyte distribution. Deprivation of sodium did not alter the volume or distribution of body water

TABLE VIII

The distribution of sodium and potassium in patients with chronic renal insufficiency

Patient	Regimen*	Body wt.	Total body water	Extracell. fluid	Intracell.† fluid	Plasma Na	Total Na	Extracell. Na	Intracell. Na	Intracell. Na conc.	Plasma K	Total K	Extracell. K	Intracell. K	Intracell. K conc.
		Kg.		*Liters*		*mEq./L.*		*mEq.*		*mEq./L. "cell" water*	*mEq./L.*		*mEq.*		*mEq./L. "cell" water*
C. P.	Control	82.0	40.9	16.5	24.4	135	2,870	2,227	643	26	3.8	2,633	63	2,570	105
T. C.	Control	61.6	30.7	15.5	15.2	138	2,475	2,140	335	22	5.0	2,355	78	2,277	150
	Na depriv.	60.8	30.1	14.9	15.2	137	2,440	2,040	400	26	4.1	2,410	61	2,349	155
	Repletion	60.9	31.1	16.1	15.0	140	2,885	2,253	632	42	4.7	2,535	76	2,459	164
J. F.	Repletion	51.4	30.7	14.9	15.8	136	2,478	2,028	450	28	5.0	1,962	75	1,887	120

* Regimen as noted under Table II.

† The term "intracellular" as used here must include a portion of the fluid and electrolytes within the bones since it is probable that inulin does not readily diffuse into this "space" during the time allotted for equilibration.

in any consistent manner. Body weight did not change or it decreased by no more than 1.6 Kg. (J. F.—Table III). Total body water (Table VI) remained more or less unchanged in two (J. F. and T. C.), rose in one (C. P.), and fell in two (G. B. and M. B.). Likewise the inulin "space" rose slightly in two (M. B. and G. B.) and fell somewhat in the remainder. In T. C. (Table VII) the four-hour radiosodium "space" and inulin "space" behaved similarly in showing a slight diminution of the same magnitude. The blood volume remained unaltered although the hematocrit fell in three subjects. (It might be added here that arterial pressures and pulse rates did not change and all patients remained well and capable of adjusting normally to positional shifts.)

The "total exchangeable" sodium and potassium in T. C. (Table VIII) were not affected significantly by sodium deprivation although the balance study (Table IV) revealed a net loss of 28.8 mEq. of sodium in close agreement with the decrement of 35 mEq. in "total exchangeable" sodium.

Despite the uncertainties of the methods employed it seems probable that the agreement between values obtained before and after sodium deprivation indicates relatively little real change in body water volume or distribution during deprivation. The fact that sodium repletion resulted in sizeable increments in the inulin "space" in C. P., J. F., and T. C. strengthens the view that this method is capable of indicating at least the relative magnitude and direction of change in extracellular fluid volume. And, since calculation of intracellular (and bone) electrolyte concentration depends on this figure, sodium concentration within the "cells" probably changed very little during sodium deprivation.

DISCUSSION

Sodium deprivation without depletion produces effects in patients with chronic renal insufficiency that differ qualitatively as well as quantitatively from those observed in normal persons. In four of five uremic patients the glomerular filtration rate decreased markedly in association with a fall in effective renal plasma flow during a five day period of deprivation. In all but one the output of sodium diminished relatively more than filtration indicating that increased tubular reabsorption of sodium also played a part in the response. The reduction in filtration was attended by a slight increase in serum inorganic phosphate concentration in all and by elevations in blood urea nitrogen concentration in three. In a normal individual under the same conditions urinary sodium output was far more drastically curtailed by increased tubular reabsorption alone. Even when normal persons are subjected to sodium depletion as in one subject of this study and in 17 studies by Wiggins and his associates (7), tubular reabsorption increases with little or no change in filtration. In large part this difference in response is traceable to the underlying abnormality in renal structure and function.

Uremia in chronic disorders of the kidney develops primarily on the basis of impaired filtration (9). Derangements in renal hemodynamics as well as destruction of tubules and glomeruli contribute to this defect. Hypofiltration is associated with what appears at first sight to be an even more serious disorder of tubular function. Reabsorption is greatly reduced permitting the escape of a much larger percentage of the filtered water and electrolytes than in the normal. Indeed, as in five of the eight patients examined in this study (Table I) the output of potassium may be several times in excess of the filtered load, indicating tubular secretion. Osmotic nephron diuresis as a result of the elevation in the plasma concentration of urea and other osmotically active substances may play a role in causing water and electrolyte loss. Mudge, Foulks, and Gilman (32) have shown that as much as 50 per cent of the filtered water (V/C_{in}) may be excreted in the urine in dogs receiving large amounts of urea by infusion. In only one of the subjects in this series (Table I) did V/C_{in} exceed 50 per cent and here the figure may be erroneously high owing to the low value for inulin clearance. Thus, the factor of osmotic diuresis seems a more likely cause for high values of V/C_{in} than the secretion of water by the tubules which Platt (33) has postulated.

This change in tubular activity is not to be viewed solely as the consequence of tubular destruction and glomerulo-tubular imbalance. Plasma electrolyte composition may be maintained within normal limits for years, as in some of the patients of this series (Table III) and it must be postulated that tubular and glomerular function

are approximately integrated in some manner to serve the needs of chemical regulation. Thus the alterations in tubular function appear to be related to alterations in glomerular filtration; the data in Tables I to III suggest that reabsorption diminishes roughly in proportion to filtration with advance in the disease process and a fall in glomerular filtration. (This phenomenon contrasts strikingly with the tendency observed in normal individuals for reabsorption to increase relative to filtration when the filtration rate is lowered by one means or another [22]).

However, excesses or loss of water and electrolytes or the development of conditions such as congestive heart failure, hemorrhage, or vomiting unmasks or intensifies a fundamental defect in adaptation. Fixation of urinary specific gravity and reaction is a commonplace in renal insufficiency. The failure of the patients in this series to adjust to the obligatory excretion of an anion (PAH) by increasing the output of buffer (phosphate) and by lowering the urinary pH illustrates the inability of the nephrons to compensate adequately for acute change by altered tubular cellular activity. In this situation readjustments in tubular function seem to be impossible or greatly delayed and the only available corrective mechanism appears to be hemodynamic in character. Intrarenal vasoconstriction and reduction in glomerular filtration may be part of a widespread systemic circulatory response, as in shock or cardiac insufficiency, but, on the basis of the present study, it appears that such a generalized circulatory derangement detectable by present methods is not always necessary for renal vascular readjustments.

It is certainly true that disturbance in the volume and composition of body water are the rule in renal insufficiency. Although carefully selected, all five of the uremic subjects studied presented evidence of expansion of the volume of inulin and four-hour radiosodium distribution, which, with due allowance for the uncertainties of these methods, seems to indicate enlargement of the extracellular fluid volume and diminution of the intracellular (and bone) fluid. A similar change has been observed following starvation (34) where it is apparently attributable to partial replacement of lost tissue (accounting for the shrinkage in intracellular water volume) by additional interstitial fluid. Weight loss occurs commonly during renal insufficiency and it was prominent among the patients of this series. The reduction observed in intracellular (and bone) sodium concentration is not so easily explained. Since plasma sodium concentration was within normal limits in all, the sodium content of the extracellular fluid was apparently maintained at the expense of the "cells" and/or bone. This shift may have played a role in conditioning the renal functional response to sodium deprivation. Unfortunately the values yielded by the methods available to us are not only of uncertain validity but also of uncertain application. In the present case, the magnitude and uniform direction of the change observed provide reasons for accepting its validity. But information regarding the tissues chiefly affected by such a shift is of the first importance in assessing its effect on kidney function. This information is not adducible from these data because the value for intracellular sodium content applies to all the tissues and to none in particular. In any event, renal insufficiency seems capable of producing a disturbance in cellular electrolyte metabolism that is not immediately evident in an altered plasma electrolyte pattern.

Azotemia may occur following salt depletion in the absence of renal disease (1, 35) but some disturbance in cardiovascular function is nearly always detectable. Furthermore, marked changes in plasma electrolyte pattern usually develop. Both of these factors may operate to stimulate the kidney directly either by changing the pressure under which it is perfused, by exciting generalized compensatory cardiovascular adjustments in which the renal vasculature participates, or by direct action of altered plasma composition upon the nephrons. But none of these could be clearly defined as effective agents in causing the changes noted in the uremic subjects of this series during sodium deprivation. The arterial pressure and the blood volume remained constant throughout. No evidence of any change in the volume or distribution of body water was detectable and there was no remarkable change in plasma composition apart from the elevation in blood urea nitrogen and inorganic phosphate concentrations. Nevertheless, the plasma sodium concentration did decrease slightly in all and apparently there may have been a very small decrement in total sodium mass, to judge by one balance study and determi-

nations of "total exchangeable" sodium in one individual. It is possibly this change, as small as it is, that sets in motion a reversible intrarenal vasoconstriction and subsequent diminution in glomerular filtration that gives rise to *L'azotémie par manque de sel.*

Evidently the same factors operate in the abnormal kidney as in the normal. Under the circumstances of sodium deprivation tubular reabsorption increases but to such a limited extent that dependence on this alone would not provide an adequate response to the need for sodium conservation. In the normal the response of the tubules is entirely adequate but the question may properly be raised whether a change in filtration of some kind does not also occur. This question has been much debated (22, 36) but lacks as yet any conclusive answer because it may be argued that the change required to be effective is too small for detection by the best methods now available. The increased importance of such a mechanism in the uremic patient may speak for its activity in the normal.

SUMMARY

Diminished urinary sodium excretion follows sodium deprivation without depletion by low sodium intake and small doses of a cation exchange resin (60 gm. per day). In five patients with chronic renal insufficiency secondary to chronic renal disease this response was attributable chiefly to diminished glomerular filtration (inulin clearance) apparently resulting from intrarenal vasoconstriction (a fall in PAH clearance) whereas increased tubular reabsorption alone was responsible in one normal subject.

In eight uremic patients, the fractions of filtered sodium, chloride, and water excreted in the urine under resting conditions were found to exceed the maximal values observed in normal men during imposition of loads of these substances. This "nephron diuresis" was associated, in five of the eight, with the excretion of a larger amount of potassium than could be accounted for by filtration alone, indicating tubular secretion of potassium. A study of electrolyte excretion during PAH loading in five revealed a fixation of urinary pH and inability to meet the need for obligatory excretion of an anion (PAH) by increased output of phosphate as a buffer, illustrating the lack of tubular responsivity to need.

Five uremic subjects in whom the volumes of distribution of antipyrine, inulin and T-1824 were determined presented evidence of an expansion of extracellular water (*i.e.*, the volumes of distribution of both radiosodium four hours after administration and inulin were well above normal in all) at the expense of intracellular fluid volume, possibly as a result of inanition and loss of tissue. Using values for "total exchangeable" sodium and potassium obtained in three of these, calculation of intracellular sodium content yielded values well below the normal range.

Although these findings provide an explanation for the need to conserve sodium and for the particular renal mechanism available for the purpose, they shed no light on the manner in which the renal reaction was set in motion. No change in blood pressure, blood volume or the volumes and distribution of body fluids was evident. The blood urea nitrogen content rose in three individuals, the serum phosphate concentration increased in all, and serum sodium concentration decreased slightly. According to measurements of sodium balance and "total exchangeable" sodium in one patient, 28.8 to 35 mEq. of sodium were lost during the five day period of deprivation.

ACKNOWLEDGMENTS

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CLINICAL STUDIES ON VERATRUM ALKALOIDS. III. THE EFFECT OF PROTOVERATRINE ON RENAL FUNCTION IN MAN [1]

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Recent clinical interest in the hypotensive properties of mixtures (1) as well as pure substances from veratrum (2–4) has made it desirable to study the effects of this hypotension on kidney function. Protoveratrine, which was isolated from *Veratrum album* by Salzberger in 1890 (5), was the first pure alkaloid from veratrum shown to have hypotensive properties in man without significant side effects (2).

Since the hypotension induced by protoveratrine is largely the result of a neurogenic vasodilatation (6–10), it appeared important to determine whether the kidney shared in this, and if so, whether directly as a result of neurogenic vasodilatation within it or indirectly as a result of the systemic hypotension. Freis and his associates (11) have studied renal function by clearance methods in ten hypertensive patients who received "veratrone" parenterally or "vertavis" orally. These agents are proprietary preparations from *Veratrum viride*, containing mixtures of alkaloids and standardized by biological methods. Since they contain substances of varying hypotensive properties and produce side effects (2, 12–14) it is desirable to determine the effects on renal function of a single pure alkaloid.

The administration of protoveratrine by various routes offers a method for the controlled lowering of blood pressure (4) during which time physiological measurements of renal function (or cardiac output, cerebral blood flow, peripheral blood flow, and so forth) may be made.

This report is concerned with the effect of protoveratrine-induced hypotension on glomerular filtration rate, effective renal blood flow and renal resistance, in hypertensive patients with and without chronic renal disease, as well as in some normal individuals.

METHOD

We have studied renal function 43 times in 36 subjects using familiar clearance technics (15). The subjects were adults from the wards or Out-Patient Department of the Beth Israel Hospital. Twenty-six had essential hypertension, two in the malignant phase; four had documented chronic glomerulonephritis with hypertension; one had Cushing's disease due to carcinoma of the adrenal cortex; one had severe toxemia of pregnancy; four were normal individuals.

Three experiments were carried out in patients who received a single intravenous injection of a hypotensive dose of protoveratrine. Eight studies were made utilizing the intramuscular route. In 32 studies the hypotensive level was maintained at an essentially steady state either by intermittent intravenous injections (*e.g.*, 10 to 30 micrograms every 10 to 30 minutes) or by continuous intravenous infusion of dilute solutions (of the order of 1 microgram per minute). In 17 of this last group of 32, the blood pressure was lowered acutely by an intravenous injection of 100 to 140 micrograms; in 15 it was lowered slowly by infusing protoveratrine at rates of 2.4 to 3.7 micrograms per minute for 20 to 60 minutes. Blood pressure measurements were made at intervals of 2 to 10 minutes by the auscultatory method.

Glomerular filtration rate (GFR) was usually determined by the clearance of inulin,[2] and in a few instances by mannitol [3] and sodium thiosulfate (Sulfactol [4]). Effective renal plasma flow (ERPF) was measured by the clearance of sodium para-amino-hippurate. Inulin was measured by the method of Roe, Epstein, and Goldstein (16), mannitol according to Smith, Finkelstein, and Smith (17), sodium thiosulfate according to Gilman, Philips, and Koelle (18) and para-amino-hippurate by the method of Bratton and Marshall (19). All analyses were performed

[1] This investigation was supported by a research grant (PHS-H-121) from the National Heart Institute of the National Institutes of Health, Public Health Service. An abstract of this material appeared in J. Clin. Invest., 30, 659, 1951.

[2] Generously supplied by U. S. Standard Products Co., Woodworth, Wisconsin.

[3] Generously supplied by Sharp & Dohme, Philadelphia, Pa.

[4] Generously supplied by Winthrop Chemical Co., New York, N. Y.

in duplicate. Values for GFR and ERPF were corrected to a body surface area of 1.73 square meters.

The substances for clearance were administered in saline solution (except for one study with intramuscular protoveratrine in which five per cent glucose was used) by an electrically-driven continuous infusion apparatus. Venous blood samples were drawn at the mid-point of each period, with heparin (Liquaemin sodium[5]) used as an anticoagulant. Urine collection was through a catheter, and the bladder was rinsed with water and air. In some instances 100 to 200 cc. of water was ingested hourly to insure a good urine flow. The first clearance period was begun 30 to 40 minutes after the start of the sustaining infusion. Three control periods (in two instances two periods) of 20 minutes each preceded administration of protoveratrine. Thereafter, clearances were measured for approximately one and one-half hours after single intravenous injection of protoveratrine and for two hours after intramuscular injection. In the 32 experiments in which the hypotension was maintained at a steady low level observations were continued for two to four and one-half hours of hypotension. Clearance periods were usually 30 to 40 minutes long when urine flow was diminished.

Resistances at the afferent and efferent arterioles were calculated according to the formulae of Lamport (20) and of Gómez (21). Mean blood pressure in the normotensive range was taken as one-half the sum of systolic and diastolic pressures. During hypertensive periods the mean blood pressure was taken as the sum of diastolic and 0.4 of the pulse-pressure, to minimize the effects of sharply peaked pulse-waves. Renal resistance units (RRU) were calculated as $\frac{\text{mean blood pressure (mm. Hg)}}{\text{effective renal blood flow (cc. per min.)}}$.

RESULTS

1. *Single intravenous injections of protoveratrine*

In these three experiments there was an abrupt fall in both GFR and ERPF. At the time of maximum hypotension when the mean blood pressure had fallen 24 per cent (range 18 to 28 per cent) the average change in GFR was − 28 per cent (range − 18 to − 42 per cent), the average change in ERPF at this time was − 25 per cent (range − 18 to − 33 per cent). As the blood pressure rose toward control levels GFR and ERPF tended to rise also, the latter back to initial levels. GFR, however, often remained below control values. The final average values of GFR and ERPF, when the blood pressure had almost regained control levels, were reduced by 20 per cent and 11 per cent, respectively. There was a slight tendency for the filtration fraction to decrease. These results are in contrast to those in a preliminary report of Hoobler (3) who found that renal blood flow was unchanged or slightly increased and glomerular filtration (creatinine or mannitol clearance) unchanged or slightly decreased in similar studies.

In view of the large degree of autonomous control exercised by the kidney (22), it appeared not unlikely that the acute hypotension (from a single large intravenous injection of protoveratrine) might have produced changes in renal function which were modifying any effects from a specific vasodilation due to the drug. Accordingly, experiments utilizing a more gradual fall in blood pressure, as with intramuscular protoveratrine, were made.

2. *Single intramuscular injections of protoveratrine*

The blood pressure fall after intramuscular administration occurs slowly over 30 to 45 minutes, after which it rises in several hours to control levels (4). In these eight experiments the fall of GFR and ERPF was often less than with single intravenous injection and the recovery in function began before the blood pressure had regained control values. The results resemble those obtained by Freis and his associates (11) using veratrone. The average change in GFR during the period of maximum hypotension was − 15 per cent (range + 5 to − 53 per cent) and in ERPF − 8 per cent (range + 13 to − 24 per cent). The final values, when the blood pressure level had almost regained control levels, showed a diminution in GFR of − 7 per cent (range + 16 to − 44 per cent) and in ERPF of − 1 per cent (range + 13 to − 18 per cent).

3. *Continuous or intermittent intravenous infusion of protoveratrine*

The comparison of renal function in the hypertensive state with that at lowered blood pressure without regard to fluctuating levels of blood pressure necessitated measurements made while the arterial pressure was maintained at a steady low level for prolonged periods (4). The patients studied under these conditions[6] included four nor-

[5] Generously supplied by Organon, Inc., Orange, New Jersey.

[6] In all of these GFR was measured by clearance of inulin.

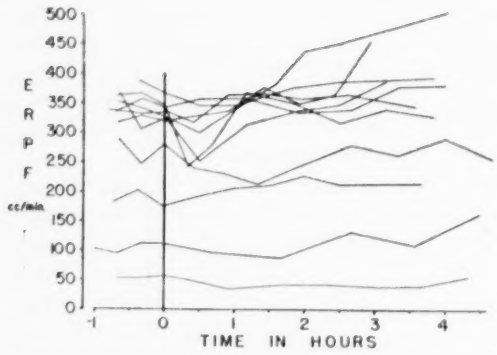

FIG. 1. THE VARIATIONS OF EFFECTIVE RENAL PLASMA FLOW (ERPF) BEFORE AND AFTER PROTOVERATRINE-HYPOTENSION IN ELEVEN HYPERTENSIVE PATIENTS

The upper seven had essential hypertension, the next below had chronic nephritis and the next malignant hypertension. The two lowest had chronic glomerulonephritis, one in the malignant phase. At 0 time the blood pressure was lowered with protoveratrine, usually to or near normal levels and maintained at a steady state throughout the remainder of the experiment.

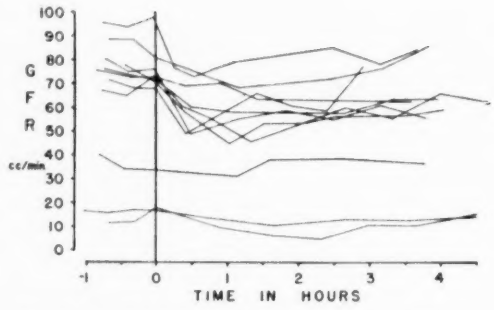

FIG. 2. THE VARIATIONS OF GLOMERULAR FILTRATION RATE (GFR) BEFORE AND AFTER PROTOVERATRINE-HYPOTENSION IN THE SAME PATIENTS AS FIGURE 1

The upper eight include seven with essential hypertension and one with chronic nephritis. The next below is a patient with essential hypertension in the malignant phase. The two lowest are patients with chronic glomerulonephritis, one in the malignant phase.

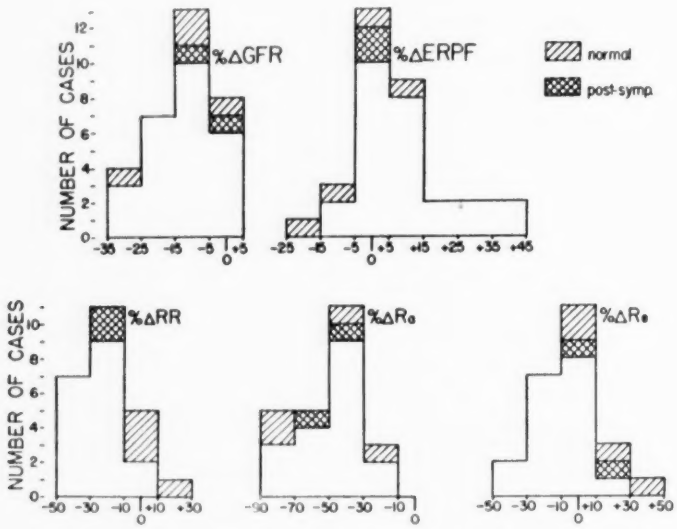

FIG. 3. THE DISTRIBUTION OF THE DATA FOR THE PER CENT CHANGES IN GLOMERULAR FILTRATION RATE (%ΔGFR), EFFECTIVE RENAL PLASMA FLOW (%ΔERPF), RENAL RESISTANCE (%ΔRR), GLOMERULAR AFFERENT ARTERIOLAR RESISTANCE—LAMPORT (%ΔRa), AND GLOMERULAR EFFERENT ARTERIOLAR RESISTANCE—LAMPORT (%ΔRe) DURING HYPOTENSION MAINTAINED AT A STEADY LEVEL FOR AT LEAST TWO HOURS

Two patients previously treated with bilateral sympathectomy from T-8 to L-2 are shown in the cross-hatched areas. Four normal individuals are shown in the hatched areas.

motensive subjects and two patients who had had thoraco-lumbar sympathectomy. In these experiments an average fall in mean blood pressure of 28 per cent (range 19 to 40 per cent) occurred. The changes in ERPF and GFR for eleven of the unoperated hypertensive patients, in whom the hypotension was maintained at a steady low level for at least four hours, are illustrated in Figures 1 and 2. These eleven patients included individuals with ERPF ranging from 50 to almost 400 cc. per minute and were typical of the entire group.

The average change in ERPF coincident with the initial fall in blood pressure was − 8 per cent (range + 8 to − 32 per cent) for the 32 experiments. Decrease in ERPF was usually more marked if the blood pressure fall had been abrupt. If the hypotension was induced slowly, *e.g.*, in 60

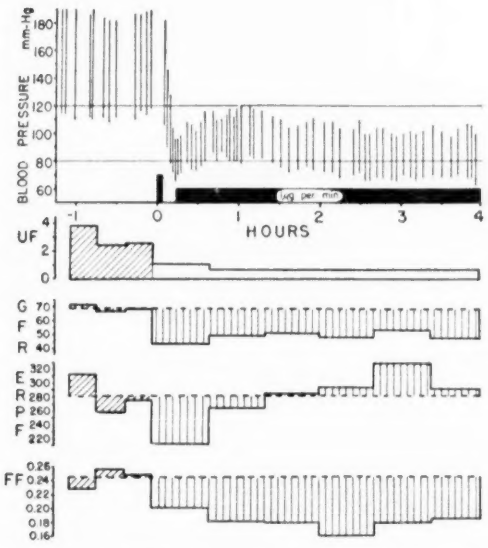

FIG. 4. A TYPICAL EXPERIMENT IN A 50 YEAR OLD WOMAN WITH ESSENTIAL HYPERTENSION

The urine was free of albumin. At 0 time 100 μg. of protoveratrine was given intravenously. After a delay of 15 minutes a continuous infusion at the rate of 1 μg./min. of protoveratrine was begun and maintained for the duration of the experiment. A typical decrease in urine flow (UF) appeared. The fall in glomerular filtration rate (GFR) was also typical. GFR did not regain control values even after four hours. Effective renal plasma flow (ERPF) which fell initially, reached control values in about one hour. The filtration fraction (FF) was diminished throughout the period of hypotension.

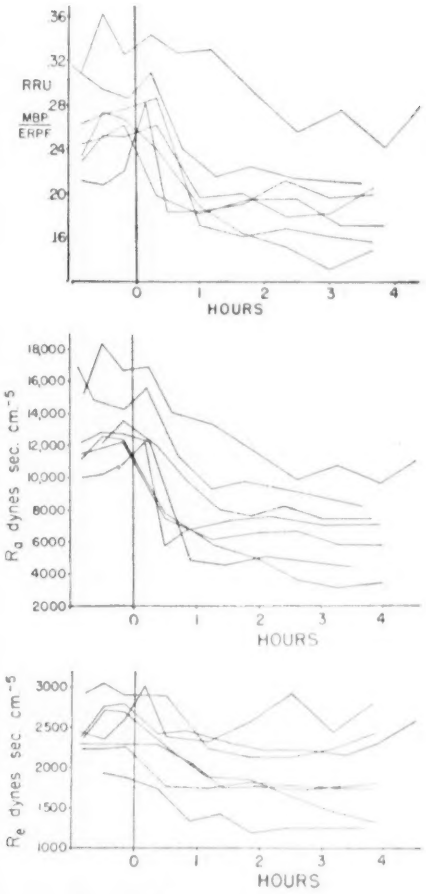

FIG. 5. THE VARIATIONS OF RENAL RESISTANCE UNITS (RRU) [MEAN BLOOD PRESSURE IN MM. HG/EFFECTIVE RENAL PLASMA FLOW IN CC./MIN.], AFFERENT RESISTANCE—GÓMEZ (Ra) AND EFFERENT RESISTANCE—GÓMEZ (Re) IN SEVEN HYPERTENSIVE PATIENTS BEFORE AND AFTER LOWERING THE BLOOD PRESSURE WITH PROTOVERATRINE

During the four hours of the hypotensive period the blood pressure was maintained at a steady lowered level (see Table I and text).

minutes, ERPF often remained unchanged. Recovery to control values of ERPF took place in one to one and one-half hours. Thereafter, ERPF remained at these levels or rose considerably above control values although the blood pressure had been maintained at a steady low level throughout this time. The average change in ERPF during

TABLE I

Pt.	BSA	Hct.	Admin. of proto.	Period	GFR_c	$ERPF_c$	B.P. range	H.R.	U.F.
				min.	*cc./min.*	*cc./min.*	*mm. Hg*	*per min.*	*cc./min.*
C. Mc.	1.62	48.5		21	96.0	362	194–202/114–116	88–90	3.04
Age 35				20	94.2	367	192–200/110–118	84–90	1.40
Sex F				20	97.9	347	194–198/114–120	84–90	3.10
			0.12 mg. →	19	76.9	241	126–202/82–112	80–90	4.74
Ess. Hypert.			1 μg./min.	22	72.7	277	110–134/74–94	74–80	0.32
				40	79.1	365	128–138/88–94	80–84	0.45
				42	81.8	349	126–144/84–90	80	0.52
				40	84.1	314	126–144/80–90	74–76	0.53
				40	77.0	338	126–136/80–90	76	0.60
				40	82.8	324	126–136/84–86	72	0.61
O. M.	1.56	36.2		20	88.7	352	174–184/118–126	80–86	7.30
Age 36				20	88.7	342	170–194/118–128	80	7.15
Sex F				20	80.3	326	174–178/110–120	84	5.70
			3.7 μg./min. ↓	40	72.6	287	134–178/84–116	72–92	3.20
Ess. Hypert.			0.5 μg./min. ↓	40	68.6	352	106–120/68–76	68–72	0.75
			1 μg./min.	40	70.1	376	106–120/70–80	72–74	0.63
				40	72.2	385	114–122/70–84	68–72	0.73
			0.5 μg./min.	40	75.5	385	108–116/66–80	64–68	0.73
				40	84.7	390	106–116/74–80	62–66	0.75
R. L.	1.54	43.0		20	83.2	368	186–190/110–114	70–72	3.85
Age 50				21	78.0	304	182–184/108–112	69–70	2.38
Sex F				20	80.5	324	186–190/110–114	72	2.55
			0.10 mg. →	42	50.8	250	96–188/66–118	56–72	1.12
Ess. Hypert.			1 μg./min.	41	57.4	312	110–116/80–88	60–64	0.66
				40	60.5	334	106–112/72–78	60–66	0.58
				40	56.0	346	100–110/66–72	60–64	0.55
				42	62.3	386	98–104/66–72	56–68	0.57
				36	55.5	342	94–106/62–70	62–68	0.56
A. O.	1.82	44.2		21	77.9	389	218–228/128–132	68	14.29
Age 47				21	71.6	368	224–228/134–136	68	12.10
Sex F			0.20 mg. →	40	57.4	343	168–232/102–136	54–68	3.95
Ess. Hypert.			0.03 mg.×7	30	44.9	346	154–170/96–104	52–57	1.00
				30	52.9	380	148–154/90–98	54–56	0.73
				30	51.8	436	156–164/98–100	56–60	0.93
				32	55.0	487	168–178/100–104	56–62	1.52
				60	55.4	474	158–164/96–100	54–57	1.43
				30	60.1	500	160–174/98–102	54–60	1.57
I. R.	1.58	39.2		23	76.5	364	182–192/116–118	69	3.78
Age 45				21	73.9	357	180–194/116	69	3.52
Sex F				20	71.5	342	190–194/116–118	72–68	3.45
			0.14 mg. →	40	60.2	354	116–194/78–120	56–76	1.25
Ess. Hypert.			1 μg./min.	40	58.3	356	126–136/80–86	58–60	0.45
				50	57.4	332	124–132/80–84	56–64	0.46
				40	57.3	338	126–132/80–84	60	0.44
				39	63.1	376	124–128/82–84	56	0.44
				40	62.4	377	128–130/80	54–56	0.48
M. R.	1.68	41.4		28	76.0	339	216–278/118–158	90–104	8.40
Age 56				30	72.8	331	206–240/120–130	84–92	6.07
Sex F				14	73.2	332	212–218/124–126	88	4.71
			2.8 μg./min. ↓	30	69.5	316	210–234/120–134	80–94	2.70
Ess. Hypert.			1.6 μg./min.	30	72.9	361	170–222/100–124	78–84	1.80
				32	63.4	363	156–184/92–104	74–84	0.75
				33	62.5	354	150–180/92–106	78	0.91
				48	62.4	363	158–172/92–104	76–84	0.85
				68	61.9	341	150–162/84–94	76–80	0.71
A. A.	1.82	43.1		20	68.6	317	222–228/130–136	66–70	3.45
Age 47				20	64.6	328	216–234/130–132	68–70	2.85
Sex F				20	64.3	320	214–218/128–130	70–72	1.80
			0.12 mg. →	30	45.5	326	184–234/112–136	64–72	1.20
Ess. Hypert.			20–30 μg.×9	30	55.1	335	190–202/110–120	60–68	0.88
				30	62.3	374	168–184/100–108	57–64	1.00
				30	57.0	345	154–172/94–106	55–62	0.67
				30	54.2	363	150–168/94–100	56–60	0.53
				30	74.6	451	160–196/96–106	54–64	0.73

TABLE I—*Continued*

Pt.	BSA	Hct.	Admin. of proto.	Period	GFR_e	$ERPF_e$	B.P. range	H.R.	U.F.
				min.	*cc./min.*	*cc./min.*	*mm. Hg*	*per min.*	*cc./min.*
H. F.	1.85	43.1		20	67.6	288	198–214/120–130	60–66	8.60
Age 39				20	65.0	245	194–208/128–130	64–70	5.40
Sex F			0.14 mg. →	21	74.2	278	198–210/128–130	66–70	3.81
			0.01–0.03 mg.	31	58.7	238	170–216/116–130	60–74	2.77
Chronic Gn.				22	55.4	230	166–178/108–120	60–64	1.41
				42	45.8	210	138–152/88–100	57–60	0.93
			i.v. 19×	41	52.1	247	140–156/92–98	54–64	0.78
				40	58.8	277	140–152/90–92	56–62	0.83
				39	54.2	260	136–148/90–96	54–58	0.90
				41	63.7	289	140–150/88–95	51–60	1.17
			↓	40	61.0	251	136–150/88–96	56–60	1.20
A. M.	1.23	42.5		20	41.1	181	184–196/108–110	68–70	5.50
Age 37				22	34.1	201	194–208/108–114	67–68	5.09
Sex F			0.09 mg. →	23	33.4	174	194–198/106–108	68–72	3.74
				44	32.5	191	116–198/82–112	50–64	1.75
Malig. Hypert.			1 μg./min.	30	31.0	207	146–166/84–90	60–66	1.33
				30	37.5	211	142–154/84–86	54–60	1.53
				39	38.0	227	132–154/78–86	56–58	1.53
				30	38.0	211	140–156/84–88	56	1.15
				38	37.2	211	130–172/76–102	52–62	1.29
			↓	37	35.9	212	140–156/82–84	52–56	1.14
A. P.	1.98	42.4		21	16.9	101	186/128	60	2.57
Age 35				20	15.7	94	190–206/130–144	64	2.75
Sex M				20	17.1	110	198–200/134–138	66	3.10
				21	17.2	110	184–200/132		3.71
Chronic Gn.			3 μg./min. ↓	60	13.6	96	172–196/118–140	52–60	2.53
			1 μg./min.	60	10.5	85	120–150/80–102	45–48	0.40
				60	13.0	131	128–136/84–90	45–48	1.27
				48	12.6	108	130–134/86–90	44	1.73
			↓	62	13.8	162	130–136/86–90	48	2.39
E. P.	1.45	38.0		20	11.5	52.3	210–260/140–148	92–104	2.95
Age 48				20	11.9	52.1	246–278/138–160	84–116	3.10
Sex F			0.11 mg.	20	17.3	54.6	238–246/130–136	80	4.02
			atropine →	21	14.4	50.3	228–232/128–134	76	2.91
Chronic Gn.			0.2 mg.	48	9.7	36.3	122–246/70–140	50–80	0.96
			1 μg./min.	42	6.1	41.5	126–148/74–88	52–60	0.64
				40	4.6	41.9	124–134/76–80	50–52	0.50
				40	10.5	38.8	130–138/76–80	50–56	0.50
				40	10.5	39.0	126–140/70–76	50–56	0.50
			↓	40	14.5	55.0	132–140/70–78	52–55	0.68
A. O.	1.82	38.6		20	57.4	336	190–208/122–128	70–76	4.70
Age 47				20	63.4	377	194–208/120–128	72–74	4.40
Sex F			0.13 mg. →	20	61.4	362	208–212/126–130	70–76	3.50
			0.02 mg.×16	40	50.3	326	152–210/92–126	56–72	1.20
Ess. Hypert.				40	55.7	376	150–164/96–100	56–60	0.73
Post. Symp.				40	53.1	350	160–174/98–108	56–60	0.88
				40	55.6	358	156–180/94–108	56–64	0.73
				40	55.7	357	158–170/98–102	56–60	1.00
				37	60.0	368	158–168/94–102	56	1.14
			↓	30	54.7	363	162–168/100–104	56–60	1.13
M. B.	1.63	38.2		20	78.4	415	198–204/112–128	60–70	3.90
Age 42				20	74.6	385	210–222/120–130	60–66	6.80
Sex F			0.11 mg. →	20	76.7	385	204–222/122–124	62–64	6.35
			1 μg./min.	40	66.0	359	150–218/90–126	52–62	1.60
Ess. Hypert.				40	70.8	402	150–166/90–96	52–54	0.60
Post. Symp.				41	73.6	388	144–156/88–94	48–54	0.39
				39	70.9	415	146–150/80–92	46–50	0.56
				40	79.7	388	132–144/80–86	45–48	0.45
			↓	40	73.3	347	134–140/80–86	44–48	0.35

TABLE I—*Continued*

Pt.	BSA	Hct.	Admin. of proto.	Period	GFR_e	$ERPF_e$	B.P. range	H.R.	U.F.
				min.	*cc./min.*	*cc./min.*	*mm. Hg*	*per min.*	*cc./min.*
D. Z.	1.80	40.1		20	100.	616	118–122/66–70	64–72	0.80
Age 26				20	111.	675	118–122/66	66–72	1.20
Sex M				22	90.5	601	122/68	70	2.41
			3.8 μg./min. ↓	36	73.3	557	124–126/66	70	2.00
Normal			1 μg./min. |	40	77.2	568	126/66–68	64	0.80
			|	40	79.7	574	102–108/54	54	0.65
			|	40	80.9	543	106–108/52–58	52–54	0.55
			|	40	66.0	440	106–108/50–60	54–60	0.70
			↓	40	71.2	491	106–108/50–60	50–54	0.40
M. D.	1.72	44.7		36	85.2	487	102/70	72–75	1.03
Age 39				20	84.6	575	92–106/70–76	74	1.50
Sex F				20	74.7	469	98–106/70–74	72	3.60
			3.8 μg./min. ↓	38	74.4	422	96–102/68–76	69–72	1.97
Normal			1 μg./min. ↓	43	66.5	388	84–104/46–66	62–72	0.61
				50	79.2	472	80–88/48–56	60–68	0.38
			1 μg./min. |	42	69.5	434	90–98/56–64	64–70	0.60
			|	40	80.0	518	92–96/58–60	70	1.95
			↓	31	71.1	433	90–94/56	64	3.87

Key: *BSA*—body surface area. *Hct.*—Hematocrit. *Admin. of proto.*—intravenous administration of protoveratrine. *Period min.*—duration of period in minutes. GFR_e *cc./min.* and $ERPF_e$ *cc./min.*—glomerular filtration rate and effective renal plasma flow corrected to a body surface area of 1.73 square meters. *B.P. range mm. Hg*—the highest and lowest blood pressure values recorded during the period. *H.R. per min.*—highest and lowest heart rate during the period. *U.F. cc./min.*—urine flow.

the last 80 minutes of the infusion, for the 26 experiments in which the duration of the hypotension was at least three hours, was + 9 per cent (range − 17 per cent to + 31 per cent).

Glomerular filtration rate fell promptly and strikingly (average − 22 per cent, range − 5 to − 63 per cent for the 32 studies) when the blood pressure fell. Control values of GFR were regained in only one-quarter of the studies. In the remainder, control values were not reached up to three or more hours. The average GFR during the final 80 minutes of the infusion for the 26 experiments in which the hypotension was maintained at least three hours was − 16 per cent (range − 35 to + 3 per cent).

The distribution of the percentage change in GFR and ERPF during the final 80 minutes of hypotension induced for at least two hours is indicated for the 32 studies in Figure 3. The data from the four normotensive individuals showed no significant variation from the hypertensive individuals. The data from four patients with chronic glomerulonephritis were not significantly different from those of hypertensive persons without obvious renal disease. The unshaded areas represent those with essential hypertension and four persons, not shown separately, with chronic glomerulonephritis and hypertension. The diminution in GFR and the rise in ERPF is evident. The detailed data from 15 typical experiments are given in Table I. These include the eleven shown in Figures 1 and 2, two patients with bilateral thoraco-lumbar sympathectomy and two normotensive persons. Figure 4 depicts a typical experiment.

The two patients who had had previous thoracolumbar sympathectomy (A. O., one month postoperative; M. B., one and one-half years postoperative) showed hypotensive effects from protoveratrine similar to unoperated patients, yet neither showed the usual fall and subsequent rise in GFR and ERPF. This was particularly noticeable in patient A. O. who had also been studied before sympathectomy. Previous to operation there was a decrease in GFR and a marked increase in ERPF during the hypotensive period; after operation similar decrease in blood pressure caused essentially no change in ERPF and GFR.

Urine flow. In all instances the administration of protoveratrine caused a profound diminution in urine flow (Table I), frequently to less than 1.0 cc. per minute despite the constant intravenous infusion of 1.7 to 3.0 cc. of 0.9 per cent NaCl solution per minute, and, in some instances, 100 to 200 cc. of water orally every hour. The decrease in urine flow was the result of active re-

sorption of glomerular filtrate water, as evidenced by a consistent, marked increase in the urinary concentration of inulin and PAH. Oliguria, as low as 0.5 cc. per minute was often present with normal or only moderately decreased clearances of inulin and PAH (see Table I). The mechanism of this oliguria and the excretion of sodium and potassium during it are described elsewhere (23).

Variations in resistance of the afferent and efferent glomerular arterioles

The hypotension induced by protoveratrine is accompanied by a marked fall in renal resistance units (RRU) (Figure 5). Figure 3 shows the distribution of the data from 24 experiments, including four normotensive individuals. In these patients calculations were based on the values obtained during the final 80 minutes of at least four hours of steadily maintained lowered blood pressure. The decrease in RRU was quite marked, the average fall being − 27.5 per cent. Using the formulae derived by Lamport it appeared that the major part of this decreased resistance was due to a fall in afferent glomerular arteriolar resistance (Ra), the average change in Ra being − 53 per cent. Only a small portion of the total decrease was due to diminution in efferent glomerular arteriolar resistance (Re), in which the average change was − 14 per cent.

For 15 hypertensive patients there were sufficient data to allow calculation of Ra and Re according to the formulae suggested by Gómez (21). In every instance the direction and magnitude of the changes following prolonged hypotension were similar to those obtained by Lamport's formulae. The average changes of Ra and Re (Lamport) at the end of four hours of maintained hypotension were − 46 per cent and − 10 per cent, respectively; the Gómez formulae showed decreases of − 40 per cent (Ra) and − 15 per cent (Re). However, protoveratrine-induced hypotension in four normotensive individuals gave average changes in Ra of − 57 per cent and in Re of + 13 per cent (Lamport), contrasted with Ra of − 26 per cent and Re of 0 per cent (Gómez). The Lamport formulae obviously do not apply to hypotension in normal individuals, because unusually low or even negative values for Ra may be obtained.

Calculation of Ra and Re (Gómez) for seven of the patients in Table I (C. Mc., O. M., R. L., A. O., I. R., M. R., H. F.) indicated that the decrease in Ra was abrupt and usually accompanied the blood pressure fall, reaching lowest values in about one hour (Figure 5). The decrease in Re was of smaller magnitude and in two of the seven patients insignificant. The decrease in Ra constituted the major portion of the diminution in renal resistance (Figure 5).

In the two patients who had had sympathectomy, decrease in Ra was similar to unoperated patients. Re remained unchanged.

DISCUSSION

A desirable hypotensive agent should cause a decrease in blood pressure without reducing blood flow or function in any vital part of the body. It is well known, for example, that in patients with coronary artery disease abrupt or prolonged falls in blood pressure may lead to coronary occlusion or myocardial infarction (24). However, the lowered blood pressure which results from the use of protoveratrine, at least within a certain dosage range, appears to be due primarily to a release of arteriolar vasoconstriction and diminution of total peripheral resistance. It therefore differs from the hypotension which occurs after a sudden decrease in effective circulating blood volume as in acute hemorrhage. Patients O. M. and R. L., in Table I, illustrate that marked hypotension can be maintained with protoveratrine without diminishing renal blood flow. Hoobler, Corley, and Kabza (3) have demonstrated that protoveratrine causes diminished resistance in the limb vessels in hypertensive patients. A similar decreased resistance in the cerebral vessels has been reported by Crumpton and his associates (25). Nonetheless, it still is possible that unusually prolonged and severe hypotensive action from protoveratrine might result in impaired circulation to vital areas such as heart or brain, particularly in the presence of atherosclerosis.

The degree of renal vasodilation which can be induced by protoveratrine hypotension may be a measure of the functional vasoconstriction present in the kidney. This vasodilation differs from that which occurs in hypertensive persons during fever, since the latter takes place during the period of increased cardiac output (26). Renal vasodilation after protoveratrine is not confined to es-

sential hypertension, but occurs also in chronic glomerulonephritis. In patients with chronic renal disease, even with nitrogen retention (E. P. and A. P. in Table I), maintaining a steady lowered level of blood pressure is associated with restoration of ERPF to control (hypertensive) levels, and a fall in total renal resistance. In individuals with essential hypertension, ERPF may rise considerably above control levels as the blood pressure is maintained at a steady low level (A. A. and A. O. in Table I). Patients with benign essential hypertension and PAH clearance of 300 to 400 cc. have normal extraction ratios of PAH (27–29). In these, at least, any rise in ERPF means increased blood flow. In those patients with chronic renal insufficiency in whom extraction ratios are diminished (27, 29) either a decreased in renal vasoconstriction or, what is less likely, an improvement in extraction ratio has obviously occurred as a result of the lowered pressure.

Although ERPF always recovered to or above control values during hypotension prolonged for two to four hours, this was not true of glomerular filtration rate. In only one-fourth of the patients did GFR regain control levels, the average value being diminished by 16 per cent at the end of three to four hours. For individuals whose filtration rate is at least one-half normal, such falls are of little consequence. Patients on rigid sodium restriction have similar decreases in GFR (30) without any rise in non-protein nitrogen. However, in the presence of severe nitrogen retention and very low filtration rates, a small additional fall in GFR might be sufficient to cause further rise in NPN. Actually, when administering protoveratrine by various routes to patients with nitrogen retention for as long as seven weeks, we have noted slight rises in NPN, no change, and in some cases a significant fall (31).

Gómez has suggested that the predominant change in the renal circulation in hypertension is an increase in afferent glomerular arteriolar resistance (21), rather than efferent glomerular arteriolar resistance, as has been previously thought (15, 20). Analysis of the decrease in total renal resistance using Gómez' (or Lamport's) formulae indicates that it is primarily the afferent arteriole which dilates as the systemic pressure diminishes.

The data from the two sympathectomized patients, although scanty, suggest that the denervated kidneys in essential hypertension do not respond to vasodepressor influences with variations in filtration rate and blood flow as do the normally innervated ones. This is of interest in comparison with the data of Pfeiffer and Wolff (32) which indicated that the functional response of the kidney to pressor influences was altered similarly by sympathectomy.

SUMMARY

1. Renal clearances of inulin and PAH have been studied 43 times in 36 subjects, 32 with hypertension, before and after induction of hypotension with protoveratrine.

2. Effective renal plasma flow was usually diminished (average − 8 per cent) with the acute onset of hypotension, but usually rose in one and one-half hours to control levels or higher, although the hypotension was maintained.

3. Glomerular filtration rate usually fell strikingly (average − 22 per cent) with the onset of hypotension. After three hours of steadily maintained hypotension, GFR averaged 16 per cent below control levels. At the end of four hours GFR did regain control values in one-fourth of the patients.

4. Oliguria characteristically appeared when the blood pressure was lowered. Recovery to control values of GFR or ERPF often took place despite persistence of the oliguria, which usually lasted several hours.

5. Renal vasodilation occurred in patients with essential hypertension as well as those with chronic glomerulonephritis when the blood pressure was maintained at a lowered level with protoveratrine.

6. Calculation of changes in afferent and efferent resistance using Gómez' formulae indicated that the major decrease in renal resistance during the period of hypotension occurred at the afferent glomerular arteriole. The decreases in afferent and efferent arteriolar resistance were similar whether calculated by Gómez' or Lamport's formulae.

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THE RELATIONSHIP BETWEEN THE CLEARANCE AND THE PLASMA CONCENTRATION OF INULIN IN NORMAL MAN

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INTRODUCTION

Shannon and Smith (1) found that in normal man, the rate of excretion of inulin was directly proportional to its concentration in plasma when the latter varied from 50 to 400 mg. per cent. Later Miller, Alving, and Rubin (2) extended these observations to plasma concentrations of inulin between 3 and 96 mg. per cent and reached similar conclusions. Both groups considered that this constituted important evidence that inulin clearance measured the rate of glomerular filtration. Recently, Ferguson, Olbrich, Robson, and Stewart (3) presented data showing that the rate of excretion of inulin was not directly proportional to its concentration in plasma, and that the clearance of inulin was dependent upon the concentration of this substance in plasma.

In view of the significance generally attached to the clearance of inulin in renal physiological studies and in view of the critical importance of precise estimation of glomerular filtration rate, it was decided to reinvestigate this problem.

MATERIALS, METHODS AND CALCULATIONS

One male and thirteen female subjects were selected for the study from the outpatient population of the George Washington University Hospital. None showed any evidence of cardiovascular renal disease on the basis of history, physical or laboratory examination. The group was predominately female because of the greater ease with which this type of study can be performed in women.

Clearance measurements were made in the postabsorptive state. Water was offered to the subjects to drink at the rate of about 250 ml. every 30 minutes, but was not forced if discomfort or nausea appeared. Preliminary blood and urine samples were collected for the estimation of blanks. Each experiment consisted of 3 groups of clearance periods, for each of which the plasma concentration of inulin was established and maintained at a constant value by appropriate priming and sustaining infusions. The constant levels sought were 5, 50, and 175 mg. per cent, respectively. Thirty minutes after the start of each infusion, three clearance periods were obtained. Periods varied from 10 to 20 minutes, depending on the urine flow. Urine was collected from an indwelling multi-eyed, soft rubber catheter, and each collection was terminated by washing twice with 20 ml. of distilled water followed by insufflation and re-expression of air. Blood was collected at the midpoint of each period from an indwelling femoral arterial needle and received into oiled syringes to which 2 drops of heparin solution had been added.

Inulin was measured in zinc filtrates of plasma and urine by a modification of the method of Harrison (4) after preliminary exposure to yeast. When the plasma concentration of inulin was less than 10 mg. per cent, the following procedure was used. Two ml. of plasma or urine were placed in a test tube to which 0.5 ml. of water and 0.5 ml. of a 40 per cent yeast suspension were added. Recovery experiments were made simultaneously in which 2 ml. of blank plasma, 0.5 ml. of an inulin standard and 0.5 ml. of yeast suspension were used. Fermentation was allowed to proceed for 20 minutes, following which the tubes were centrifuged and 2 ml. of the supernatant were removed. The latter was added to 5 ml. of a 4 per cent solution of zinc sulfate in N/10 H_2SO_4 and the protein was precipitated by the addition of 2 ml. of 0.75 N NaOH. From this point forward, the procedure followed that employed at high levels of inulin. In all analytical runs, recoveries were included. These averaged 98.6 per cent ($\sigma = 2\%$) over a range of from 5 to 40 mg. per cent of inulin. Blank plasma from various patients contained from 0.15 to 1.15 mg. per cent of inulinoid chromogen, averaging 0.55 mg. per cent. At plasma concentrations of inulin between 3 and 10 mg. per cent the color attributable to blank averaged 8 per cent of the total color and in only 2 of the 14 subjects did it constitute more than 10 per cent of the total color during the clearance periods at low concentrations of inulin. The inulinoid chromogen in blank urine was equivalent to between 0.00 and 0.2 mg./min. in the seven subjects examined and constituted between 0.0 and 3.1 per cent of the total color (average 1.2%) when the rate of inulin excretion was low.

Clearance was calculated by dividing the average minute rate of excretion by the midperiod plasma concentration of inulin. No correction for delay time was applied.

The data were analyzed in two ways: By relating the rate of excretion to the plasma concentration of inulin and by examining the clearance of inulin as a function of its concentration in the plasma. In such an analysis it is

necessary to consider the sources and magnitude of the errors involved. These are of two types: *1*) The errors of the chemical estimations of the concentrations in plasma and urine, and *2*) that attributable to variable emptying of the bladder, variable residual urine in dead space, and possibly to true variation in the excretion of inulin. The first type of error is, in practice, negligible with respect to the very considerable error in the collection of the urine. Further consideration also indicates that the absolute magnitude of the error is directly proportional to the absolute value of the measured quantity. Since all the chemical determinations are performed on final solutions containing approximately equal concentrations of inulin, the relative errors are approximately constant and are magnified by the preliminary dilutions to which the samples have been subjected. The absolute error is therefore proportional to the original concentration. A similar consideration applies to the error in the rate of excretion of inulin since a given fraction of the urine in any one period will contain more inulin when the rate of excretion is high than when the rate of excretion is low.

In the calculation of the regression of rate of excretion on plasma concentration, it is assumed that the error in the determination of the plasma concentration is negligible with respect to that involved in the determination of the rate of excretion; this assumption is apparently warranted. However, in the usual solution by the method of least squares it is assumed that the absolute variability does not change systematically over the range to which the data apply. This is obviously not the case in the present situation. In such an analysis of these data which cover up to a 40-fold range of UV and P, far too much weight is given to the values at high plasma concentration, since the deviations of the points at low plasma concentration from the regression line are small in any case. Furthermore, the error in the estimation of the intercept is exaggerated to an even greater extent and makes a significant departure of the intercept from zero (the hypothesis to be tested) very difficult to detect.

To overcome this distortion, the standard statistical procedure was applied by weighting each value by the inverse of its estimated variance. In this case, the variance is estimated to be proportional to the square of the absolute value. The procedure used was as follows: A line relating UV to P in each experiment was calculated by the method of least squares using the observed values of UV and P for each period. The values of the intercept on the UV axis and the standard error of the value of the intercept were also calculated. These are presented in the left-hand columns of Table III. The line thus calculated was used for the determination of weighting coefficients to be applied to the data in a further calculation. Values of UV on the line were determined corresponding to the several values of observed P. A

TABLE I

Inulin clearance at several plasma concentrations of inulin in two normal subjects

Subject	Time	Urine flow	Plasma	Inulin Excreted	Clearance	Δ from mean* C_{in}
	min.	*ml./min.*	*mg. %*	*mg./min.*	*ml./min.*	*%*
O. C.	0		Priming Infusion: Inulin 0.65 gm.			
	1		Start Sustaining Infusion: Inulin 5.5 mg./min. in 3.5 ml. saline			
	65– 87	3.14	5.66	5.65	99.7	+1.4
	–108	6.02	5.79	6.10	105.4	+7.2
	–129	3.25	5.67	5.24	92.4	−6.0
	130		Priming Infusion: Inulin 6.5 gm.			
	134		Start Sustaining Infusion: Inulin 55 mg./min. in 3.5 ml. saline			
	171–185	1.88	62.0	58.2	94.0	−4.4
	–202	3.20	58.0	60.8	104.8	+6.6
	225		Priming Infusion: Inulin 19.5 gm.			
	231		Start Sustaining Infusion: Inulin 220 mg./min. in 3.5 ml. saline			
	262–278	1.49	184.0	180.0	97.7	−0.6
	–292	3.50	178.8	167.0	93.9	−4.5
	–307	2.60	166.5	163.5	98.5	+0.2
J. B.	0		Priming Infusion: Inulin 0.6 gm.			
	1		Start Sustaining Infusion: Inulin 5 mg./min. in 3.5 ml. saline			
	55– 75	11.75	4.37	5.51	126.1	−3.3
	– 87	10.85	4.46	5.49	122.9	−6.5
	– 99	12.00	4.40	5.41	123.1	−6.3
	104		Priming Infusion: Inulin 6.0 gm.			
	108		Start Sustaining Infusion: Inulin 50 mg./min. in 3.5 ml. saline			
	137–147	5.40	51.6	64.1	124.2	−5.2
	–159	4.94	47.0	65.4	138.8	+9.4
	–172	6.15	43.3	57.1	131.8	+2.4
	177		Priming Infusion: Inulin 17.9 gm.			
	183		Start Sustaining Infusion: Inulin 150 mg./min. in 3.5 ml. saline			
	220–233	4.66	109.8	138.8	126.3	−3.1
	–245	5.52	108.8	142.7	131.1	+1.7
	–249	7.31	108.0	151.4	140.2	+10.8

* Mean of the 9 periods in each experiment.

weighting coefficient for each value of UV and P was calculated using the reciprocal of the square of the value of UV lying upon the line. The observed values of UV and P were then weighted with these coefficients and the least square solution was repeated. A new line was thus obtained in which the preponderant effect of the values at high concentrations of inulin was eliminated. Its intercept with the UV axis and the standard error of the intercept were also calculated. These are presented in the right-hand columns of Table III. The new line can be used for obtaining new weighting coefficients and the data recalculated. This was tried in several instances, but since this resulted in no further appreciable change in the intercept or its standard error, further approximations were abandoned and only the results of the first weighting are presented.

In the evaluation of clearance as a function of plasma concentration, any systematic trend should appear as a significant slope of the regression line. Since in this case UV is divided by P, the effect of those errors which are proportional to concentration is cancelled out and no weighting is necessary.

RESULTS

The details of two representative experiments appear in Table I. In the first experiment (subject O. C.) the inulin clearance varied about a mean value of 98.3 ml./min. by from + 7.2 to − 6.0 per cent and there was no obvious tendency for a systematic change in clearance as the plasma concentration of inulin varied between 6 and 180 mg. per cent. In the second experiment (subject J. B.), the clearance varied between + 10.8 and − 6.5 per cent of the mean value of 129.4 ml./min. and the clearance seems to be somewhat higher at high plasma concentrations. On statistical analysis, this trend is more nearly significant than in any other experiment, but could be expected by chance alone in from 5 to 10 per cent of cases.

Table II presents a summary of all experiments. Here the average clearance at each approximately constant plasma level of inulin is presented. With one exception (subject A. G.) the clearance is almost identical at all plasma concentrations for each subject.

The statistical data on all subjects appear in Tables III and IV. In Table III are presented the intercepts and the standard errors of the intercepts of the regression line of excretion rate on plasma concentration of inulin. Data derived from calculations before and after weighting are

TABLE II

Inulin clearance at a series of plasma concentrations of inulin. Data is average of 3 consecutive periods except where noted

Patient	Age	S. A.	Average inulin		Patient	Age	S. A.	Average inulin	
			Plasma	Clearance				Plasma	Clearance
		sq. m.	*mg. %*	*ml./min.*			*sq. m.*	*mg. %*	*ml./min.*
J. B.	25	1.65	4.4	124	E. L.	22	1.60	4.3	99
			47.3	132				49.4	108
			108.9	133				169.7	104
V. C.	35	1.67	5.9	105	J. M.	30	1.62	8.9	117
			63.9	105				54.7	115
			173.2	104				127.9	111
O. C.	38	1.62	5.7	99	L. P.	26	1.37	8.1	99
			60.0†	99†				42.3	101
			176.3	97				124.3	100
A. G.	26	1.49	9.4	129	L. R.	28	1.60	5.6	111
			52.9	124				55.7	117
			138.6	108				177.3	119
E. H.	23	1.34	4.4	78	J. W.	31	1.48	3.2	146
			35.5	84				37.2	145
			123.7	86				101.2	153
J. H.*	34	1.73	10.3	99	E. W.	29		6.0	92
			55.2	99				63.3	97
			151.6	99				179.2	90
M. J.	33	1.60	13.6	69	G. W.	27	1.56	4.0	119
			154.9	63				46.4	109
								138.4	117

* Male; all other subjects female. † Average of 2 periods; all others of 3.

TABLE III

Statistical analysis of regression of rate of excretion on plasma concentration of inulin before and after weighting

Patient	Unweighted series			Weighted series		
	Intercept	Standard error of intercept	"t"*	Intercept	Standard error of intercept	"t"*
	mg./min.	*mg./min.*		*mg./min.*	*mg./min.*	
J. B.	−0.484	2.54	0.19	−0.386	0.19	2.03
V. C.	+0.278	3.42	0.08	+0.127	0.50	0.25
O. C.†	+0.734	1.81	0.40	+0.053	0.26	0.20
A. G.	+5.160	7.69	0.67	+1.654	1.32	1.25
E. H.	+0.057	4.72	0.01	+0.270	0.36	0.75
J. H.	−0.151	2.10	0.07	+0.060	0.70	0.09
M. J.‡	+1.259	3.47	0.36	+0.985	0.53	1.86
E. L.	−0.172	6.55	0.03	+0.314	0.34	0.92
J. M.	+1.618	4.80	0.34	+0.245	0.87	0.28
L. P.	−0.077	1.01	0.08	+0.145	0.28	0.52
L. R.	−0.625	0.78	0.80	−0.419	0.39	1.07
J. W.	−2.100	24.55	0.09	−0.097	0.49	0.20
E. W.	+2.900	3.21	0.90	+0.163	0.65	0.25
G. W.	−1.250	2.38	0.53	+0.284	0.16	1.78

* For all but noted exceptions, there are 7 degrees of freedom (nine clearance periods). For p to be < 0.05, "t" must exceed 2.3; for it to be < 0.01, "t" must exceed 3.5.

† 6 degrees of freedom. For p to be < 0.05, "t" must exceed 2.4.

‡ 4 degrees of freedom. For p to be < 0.05, "t" must exceed 2.7.

included. In the unweighted series, the intercepts are small—less than 2.0 mg. per min. in 11 of 14 subjects—as frequently positive as negative, and never significantly different from zero. However, it is apparent that this analysis is, as predicted in the discussion of methods, biased. The intercepts have, relative to their own values, high standard errors, so that the probabilities, instead of being randomly distributed from high values to near significance, are all large. After weighting, the intercepts generally approach zero more closely, and only one is greater than 1.0 mg./min. The standard errors of the intercepts are reduced to a proportionately greater degree. None of the intercepts differ significantly from zero. The probabilities are more reasonably distributed in the weighted than in the unweighted series. In Table IV are presented the slopes and the standard errors of the slopes of the regression lines of clearance on plasma concentration of inulin. The slopes are positive in eight instances, negative in six, and small in all. None differs significantly from zero. The series of probabilities seem to be reasonably well distributed.

TABLE IV

Statistical analysis of regression of clearance on plasma concentration of inulin

Patient	Slope	Standard error of slope	"t"*
J. B.	+0.075	0.05	1.50
V. C.	−0.008	0.02	0.40
O. C.†	−0.016	0.02	0.80
A. G.	−0.165	0.23	0.72
E. H.	+0.040	0.07	0.57
J. H.	+0.000	0.02	0.00
M. J.‡	+0.134	0.20	0.67
E. L.	+0.002	0.05	0.04
J. M.	−0.055	0.79	0.70
L. P.	+0.006	0.10	0.06
L. R.	+0.019	0.05	0.38
J. W.	+0.090	0.16	0.56
E. W.	−0.035	0.03	1.17
G. W.	−0.005	0.04	0.13

* Same significance as in Table III.
† Same significance as in Table III.
‡ Same significance as in Table III.

DISCUSSION

Clearance measurements are subject to a certain amount of essentially inherent error. Smith (5) analyzed data from a large number of experiments of variable duration in which inulin clearance was measured by the constant infusion technique and in which no systematic trend in the clearance was evident. He found that the standard deviation about the mean clearance for an individual was 8.9 per cent, so that about 70 per cent of the periods should be within ± 8.9 per cent of the average of all periods and 96 per cent within ± 17.8 per cent of the average. He concluded that variability of this magnitude is to be expected in the routine measurement of inulin clearance. In the experiments presented in Table I, as well as in all the other experiments, the observed variability was within these limits. Most of this variability can be attributed to incomplete emptying of the bladder and to the accumulation in or washing out from the dead space of inulin as a result of an increase or decrease in urine flow. Errors due to incomplete emptying are not cumulative, but tend to cancel out in successive periods. The residual from one period is usually collected in the following period, so that the average of the two is more nearly correct. Dead space errors are likely to be acute, and if inulin is lost into or appears from dead space as a result of a change in urine flow, a steady state is soon reached. Accordingly, the average of successive periods is likely to reflect this error less than the single period in which the acute

change occurred. In fact, it may be seen from Table III that when the average of three successive periods at one plasma level of inulin is compared to the average of all (usually nine) periods for any individual, the variability is much smaller than that estimated by Smith and by others (3).

With these considerations in mind, it seems likely that the variability observed was well within the limits of error of the technique employed. It is still possible that actual fluctuations in filtration rate account for part of the variability. The data offer no evidence to support the contention that inulin clearance is dependent upon inulin plasma concentration. All of this evidence is in harmony with the conclusions drawn by Shannon and Smith (1) and by Miller, Alving, and Rubin (2).

While it is not possible to offer a definitive explanation for the differences between these observations and those of Ferguson and her associates (3), some comment and speculation seem to be in order. These authors reported a series of inulin clearance measurements following a single intravenous injection of the test substance and another series of clearance measurements at several constant plasma concentrations of inulin. The variation of clearance with plasma level of inulin is not as impressive in the second series as in the first. When subjected to the types of analysis used in this report, the results are as follows: Before weighting the intercepts calculated for each experiment did not differ significantly from zero, but when the two experiments on C. M. were treated as one, the difference between the intercept and zero was of borderline significance ($p = 0.01 - 0.05$); after weighting, the difference between the intercept and zero for both individual and the combined experiment on C. M. were of borderline significance, whereas this difference was not significant in the experiments on W. R. and J. C.

The first series of experiments in which the plasma inulin concentration was falling rapidly showed a remarkable dependence of clearance on plasma concentration, the ratio of highest to lowest clearance averaging 1.4 and reaching 2.3 in one subject. The greatest source of error in this type of experiment is the uncertainty of the correction for delay time. Recent evidence (6–8) suggests that a delay time correction of from 20 to 30 minutes may be more nearly correct than the 2½ to 6 minutes correction used by these authors. Considering the form of the curve relating plasma concentration of inulin to time after a single intravenous injection, it may be deduced that a longer delay time correction would reduce the apparent clearance at high plasma inulin levels much more than at low levels, and so reduce the dependence which they emphasize. This surmise is supported if their subjects are divided for purposes of analysis into two groups, one of 8 whose initial inulin clearance exceeds 80 ml./min., the other of 7 whose initial clearance is less than 80 ml./min. Necessarily, the plasma concentration of inulin fell less rapidly in those with lower clearances. The values of the intercepts decrease with inulin clearance and the mean intercept of the first group is significantly smaller than that of the second. In other words, Ferguson's results are most striking when the delay time error is probably largest, and least striking when the error is predictably smallest.

Systematic errors possibly inherent in both series of their experiments such as those due to chemcal methodology, to variable plasma and urine inulinoid blanks, to systematic changes in urine flow with consequent systematic dead space errors, and to inhomogeneous inulin are all impossible to evaluate in the report of Ferguson. As far as can be determined, none of these errors were important in the data herein reported.

The fact that the experiments performed by these authors at constant plasma concentrations of inulin show a dependence of clearance on plasma concentration that is at best of borderline significance, and the recognition that serious reservations must be entertained in the case of their single injection experiments, tend to reduce the area of disagreement between their data and that presented in this paper.

SUMMARY

1. The clearance of inulin has been studied in 14 normal subjects in experiments in which the concentration of inulin in plasma was held constant for 3 periods at each of three levels, about 5, 50 and 175 mg. per cent, respectively.

2. Analysis of the data indicated that within the limits of error of the method, the clearance was identical at all plasma concentrations.

3. These data support the conclusion that inulin is cleared at the level of glomerular filtration.

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COMPARISON OF THE COOMBS TEST WITH OTHER METHODS FOR BRUCELLA AGGLUTININS IN HUMAN SERUM [1]

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During the past year we have compared several techniques for testing human sera for Brucella agglutinins. These have included a tube dilution method, the rapid slide method (Huddleson) and a rapid slide or screening test designed by Castaneda (1). In addition, a blocking test and a modification of the indirect Coombs test were investigated. High speed centrifugation of Brucella-serum mixtures was also used to increase the titer of Brucella agglutinins.

Several unsuccessful attempts also were made to adapt the Middlebrook-Dubos (2) hemagglutination method to the detection of antibodies against Brucella. In addition, paper chromatograms were made in attempts to differentiate specific from non-specific Brucella agglutinins by the method of Hess and Roepke (3).

Sera from 104 adults were studied. Of these subjects, 27 had brucellosis proved by culture, 28 had presumptive evidence of brucellosis, and 49 had no evidence of infection with Brucella and were classed as "normal" controls.

METHODS

Venous blood was obtained aseptically and allowed to clot in a sterile glass tube in a refrigerator. The serum was separated within a few hours. It was not heat-inactivated but was tested immediately or stored in a deep-freeze cabinet at 0° C. until tested.

Tube dilution agglutination method. The agglutinin titer was determined by a modification of the method of Fitch, Donham, Bishop, and Boyd (4). The serum was diluted two-fold serially from 1:10 to 1:2,560 with 0.85 per cent saline solution. *Brucella abortus* antigen (0.5 ml.) was added to 0.5 ml. of saline (control) and 0.5 ml. of each dilution of the serum in separate tubes.[2] The antigen was prepared by diluting a heavy suspension of heat-killed *Br. abortus* cells to 1:100 with saline solution. The stock antigen suspension (B.A.I.) was obtained from the Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Maryland. After the addition of the antigen, the content of each tube was mixed by shaking and then incubated for 24 hours in a water bath at 37° C. The tubes were then refrigerated at 5° C. for 1 hour. They were examined immediately against a black background with mild agitation. The degree of agglutination was recorded as 0, ±, 1+, 2+, 3+, and 4+ depending on the size and number of aggregates as well as the degree of clearing of the supernate. The agglutinin titer was taken to be the highest dilution of serum showing 1+ or greater agglutination. The antibody titer as determined by all other methods was based on a comparable endpoint.

Rapid slide agglutination method. The method of Huddleson (5) was followed exactly. The serum was diluted serially from 1:20 through 1:320 by addition of Brucella antigen.[3] In those sera with a high titer of agglutinin the serum was diluted 1:16 with 0.85 per cent saline solution prior to the addition of antigen to extend the range of dilution from 1:320 to 1:5,120. The mixtures were examined at room temperature after mixing and rotation on a glass plate for three minutes.

Castaneda slide or screening test (1). *Br. abortus* killed by heat and stained with methylene blue was obtained through the courtesy of M. Ruiz Castaneda, Hospital General, Mexico City. The test was performed by two methods which proved equally satisfactory. In the great majority of cases, capillary blood was obtained from the finger tip by a small puncture with a sharp, sterile needle using aseptic technique. A large drop of blood was touched immediately upon a glass plate. A small drop of the stained antigen was added to the blood from a tuberculin syringe fitted with a 26 gauge needle. The blood and antigen were quickly mixed with a sterile needle or platinum inoculating wire. Proper proportions of blood and antigen yielded a mixture having a blue-green color. The glass plate was manually rocked in a circular fashion in the horizontal plane so as to swirl the mixed drop around gently in a depression on the surface of the glass plate. Any evidence of drying vitiated the results. Hence, the degree of agglutination was recorded within 60 seconds after mixing the blood and antigen. Complete (4+) agglutination was indicated immediately

[1] Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[2] Final dilution of the serum was carried thus to 1:5,120.

[3] Febrile *Br. abortus* antigen was obtained from Lederle Laboratories, Pearl River, N. Y.

upon rotating the plate by the appearance of a dark blue peripheral ring of agglutinated antigen separated by centrifugal force from a distinct central red zone of erythrocytes. Lesser degrees of agglutination (1+, 2+, and 3+) were indicated by slower and incomplete separation of the antigen from the erythrocytes. In a few cases where only frozen serum was available for study the serum was melted at room temperature and added to an equal volume of fresh, normal, whole human blood. The test was then carried out as described above.

"Blocking" antibodies. Only fresh, unheated sera were tested for "blocking" antibodies. The method was almost exactly the same as for the tube dilution agglutination test. After serial dilution of the suspected "blocking" serum and addition of the B.A.I. *Br. abortus* antigen, 0.03 ml. of a potent human immune serum [4] was added to each tube and a saline control tube containing no "blocking" serum. Incubation and interpretation was carried out as in the tube dilution method. Blocking was indicated by less agglutination than found in the control tube.

Coombs antiglobulin agglutination test. As with the tube dilution method, serial dilutions of the patient's serum were prepared in saline. The final volume of the diluted serum in each tube was 1 ml. One ml. of B.A.I. *Br. abortus* antigen was added to each tube. After mixing, incubation was carried out at 37° C. for 24 hours and at 5° C. for 1 hour. The tubes were then centrifuged at 4,000 r.p.m. in a Sorvall angle centrifuge for 15 minutes. The supernate was aspirated and discarded. Two ml. of 0.85 per cent saline solution was added to the sediment. After the sediment was resuspended by shaking, the tubes were again centrifuged. The washing of the sediment was carried out three times and the sediment was then resuspended in 0.5 ml. of saline. Coombs anti-human-globulin rabbit serum [5] (0.1 ml.) (6) was then added to each tube as well as a control tube with no immune serum. After mixing, incubation was carried out at 37° C. for 30 minutes. Agglutination was then recorded.

Centrifugation agglutination test. The method was exactly as described for the Coombs method with omission

[4] Tube dilution agglutination test revealed a titer of 1:10,240. The serum was stored in a deep-freeze cabinet.

[5] Obtained from Ortho Pharmaceutical Corp., Raritan, N. J.

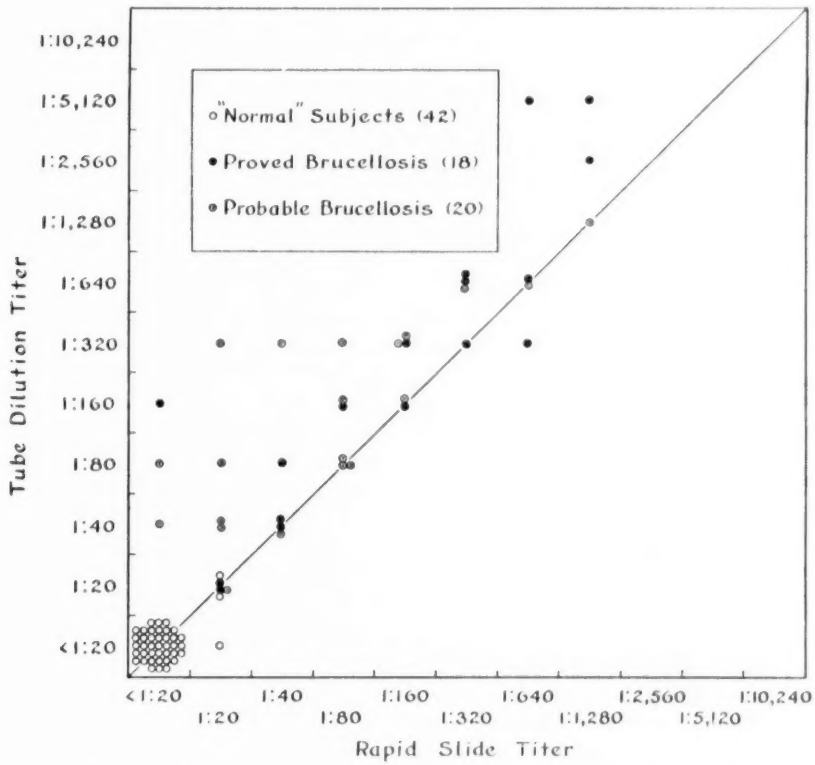

FIG. 1. BRUCELLA AGGLUTININS IN 80 SUBJECTS BY TUBE DILUTION AND HUDDLESON RAPID SLIDE METHODS

of the Coombs serum. The patient's serum was diluted serially with saline and an equal volume of B.A.I. *Br. abortus* antigen was then added. After mixing, the tubes were incubated at 37° C. for 24 hours and at 5° C. for 1 hour. They were then centrifuged in a Sorvall angle centrifuge at 4,000 r.p.m. for 15 minutes. Agglutination was recorded upon resuspension. Washing with saline proved unnecessary although it did make agglutination more definite.

Paper chromatograms. The method of Hess and Roepke (3) was used. A strip of S&S 589 filter paper ($3\frac{1}{2} \times \frac{3}{4}$ inches) was placed on a flat surface and 0.02 ml. of serum allowed to soak in at one end. The strip was hung vertically with the spot of serum immersed in 0.05 M. potassium acid phthalate (pH 6.2). After 15 minutes *Br. abortus* antigen[6] was applied along the path of migration of the phthalate developer and serum with a fine brush. The strip was then washed with 1 per cent saline using a second brush on an absorbent towel. Migration of the antibody was taken to indicate the presence of specific Brucella agglutinin. Adsorption of the antibody to the paper and failure to migrate were typical of nonspecific antibody.

[6] The antigen was hematoxylin-stained *Br. abortus* commonly used for the "ring" test for agglutinins in cream. The glycerine was removed by washing with saline. We are indebted to Dr. Martin H. Roepke, Division of Veterinary Medicine, University of Minnesota, St. Paul, for this antigen.

RESULTS

The serum of 80 subjects was tested for Brucella agglutinins by both the tube dilution and the rapid macroscopic (Huddleson) methods. The results are given in Figure 1. It was readily apparent that, with sera containing agglutinins for Brucella in a titer of 1:40 and above, the tube dilution method was more sensitive than the rapid slide method. It will be noted that five patients with brucellosis proved by culture and ten with probable brucellosis had an agglutinin titer of only 1:80 or less by either method. These patients, however, had contracted the disease at least 11 months earlier and had shown higher agglutinin titers during the first months of their illnesses. No "normal" subject was found to have

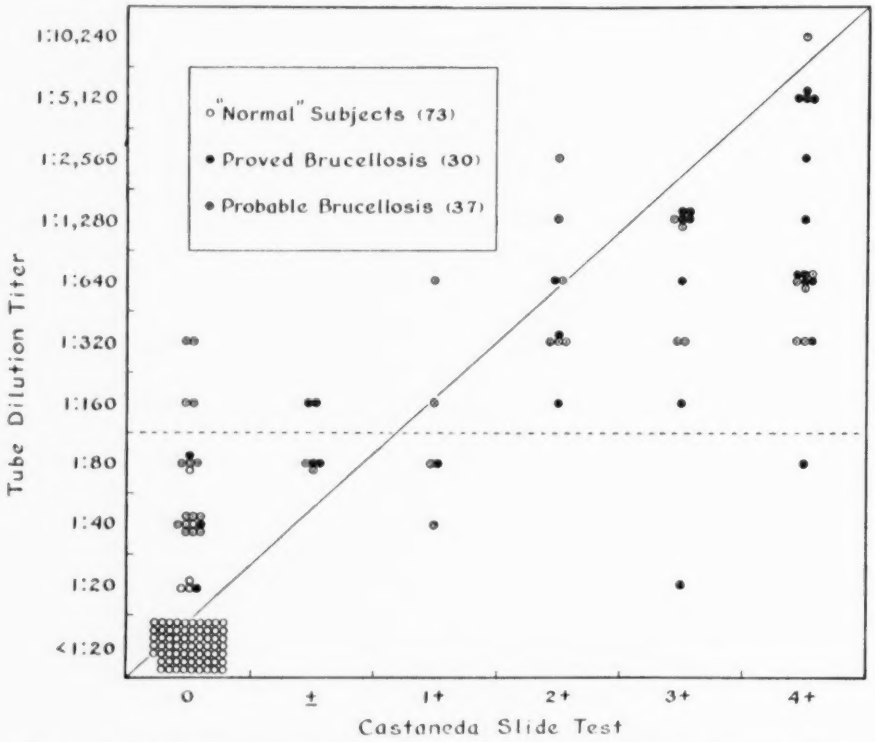

FIG. 2. THE CASTANEDA RAPID SLIDE TEST AND TUBE DILUTION BRUCELLA AGGLUTININ TITER IN 140 SUBJECTS

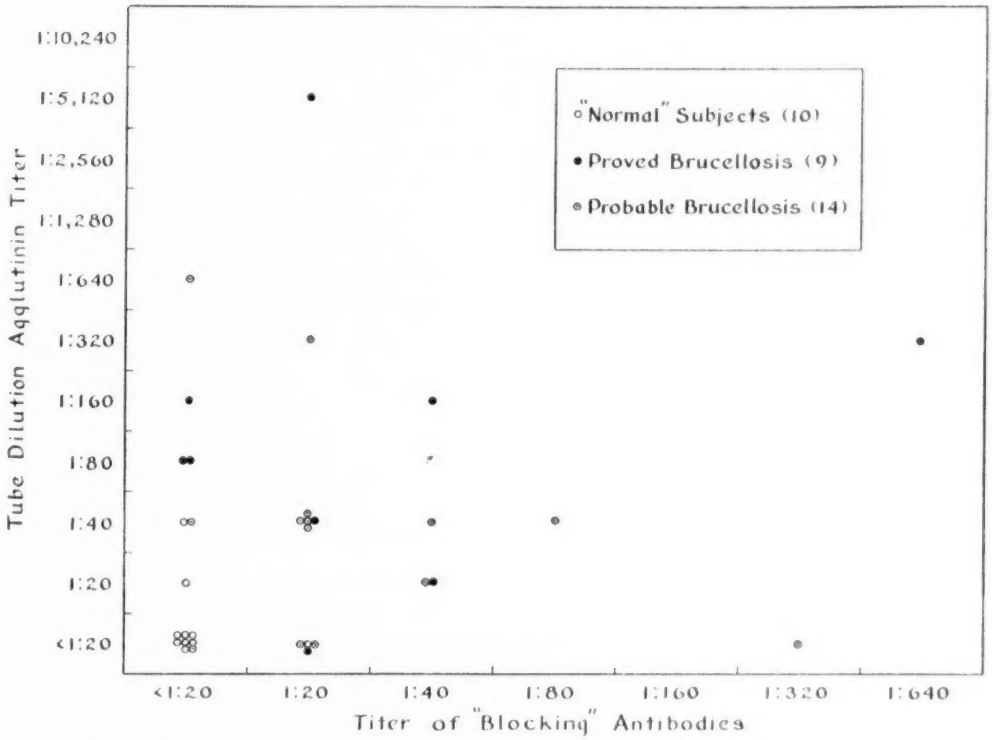

FIG. 3. THE TITER OF BRUCELLA AGGLUTININS AND "BLOCKING" ANTIBODIES IN 31 SUBJECTS

an agglutinin titer greater than 1 : 20 by either the tube dilution or rapid slide methods. With few exceptions those subjects with an agglutinin titer of 1 : 40 or greater by either method acknowledged past or present symptoms commonly found in brucellosis and admitted exposure to Brucella through ingestion of unpasteurized milk or by contact with fresh meat. The diagnosis of brucellosis had been proved by the isolation of *Br. abortus* from the blood of 16 and *Br. suis* from the blood of 2 of the 38 patients with brucellosis, in Figure 1.

There were seven patients with proved and five with probable brucellosis whose serum showed the prozone inhibition phenomenon with the tube dilution agglutination method. The prozone exceeded a titer of 1 : 20 in only three individuals. In these patients the prozone extended through 1 : 40 in two and 1 : 160 in the other. The inhibition of agglutination was usually only partial in the prozone but it was complete at the 1 : 20 dilution in three patients. The tube dilution agglutinin titer in the entire group of 12 patients ranged from 1 : 40 to 1 : 5,120 (median = 1 : 320). A definite prozone was not seen in a single instance with the macroscopic rapid slide method.

The Castaneda rapid slide or screening test for Brucella agglutinins was compared with the tube dilution titer of the serum from 140 subjects, including 30 patients proved by culture to have brucellosis and 37 with probable brucellosis (Figure 2). There was a fair correlation between the agglutinin titer and the results of the Castaneda test. There were only two individuals, both recovered from brucellosis, whose blood gave a positive Castaneda test in the absence of an agglutinin titer of 1 : 80 or higher. None of the 73 "normal" subjects had a positive Castaneda test although Brucella agglutinins were detectable by the tube dilution method in a titer of 1 : 80 or less in 5 individuals. The sole "normal" subject whose serum had an agglutinin titer of 1 : 80 had received

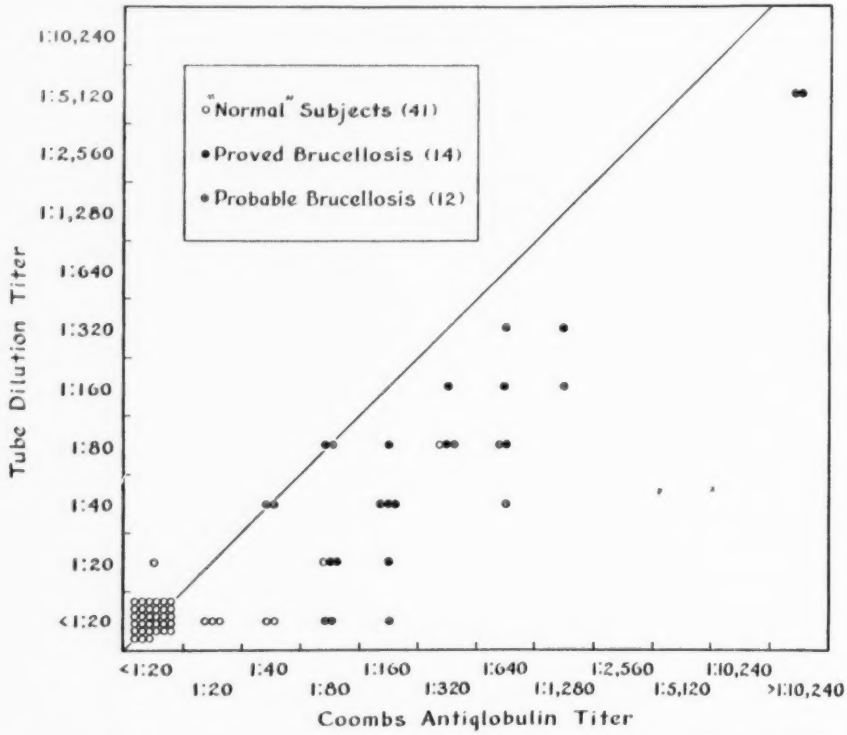

FIG. 4. THE BRUCELLA AGGLUTININ TITER IN 67 SUBJECTS BY TUBE DILUTION AND COOMBS ANTIGLOBULIN METHODS

a booster dose of 0.5 ml. cholera vaccine 6 weeks earlier. Four patients with probable brucellosis and two with bacteremic, active brucellosis did not have a definitely positive Castaneda test although their serum agglutinin titer was 1 : 160 to 1 : 320. The Castaneda test was positive with 86 per cent of 44 patients whose serum had an agglutinin titer of 1 : 160 or greater and was positive with only 5 per cent of subjects whose serum had a titer of 1 : 80 or less. Of the seven patients with proved brucellosis, whose Castaneda test was not positive, only two had active brucellosis at the time the test was done; the others had recovered from the disease. No false positive Castaneda tests were observed when correctly done with normal blood. If the blood-antigen mixtures were allowed to stand and dry, however, false "agglutination" frequently resulted.

Fresh, unheated serum was obtained from 31 individuals to be tested for "blocking" antibodies against Brucella agglutinins. Only 1 of 10 normal subjects showed any "blocking" and then only a slight suppression of agglutination in a dilution of 1 : 20 (Figure 3). Twenty-three selected patients with brucellosis were included; the diagnosis was proved by isolation of *Br. abortus* from the blood of nine. The patients were selected because their tube dilution agglutination test showed an inhibition prozone or only a low titer of agglutinins. Many of these patients had been ill for more than a year and most of them had been exposed to Brucella for many years.

There was little correlation between the titer of Brucella agglutinins and the titer of "blocking" antibodies. The titer of "blocking" antibodies exceeded 1 : 40 in only three patients. The "blocking" or inhibition of agglutination was not complete except occasionally in the lowest dilutions.

The Coombs antiglobulin method proved effective in increasing the sensitivity of the agglutination method without destroying its specificity (Figure 4). The Coombs method frequently in-

creased by fourfold or more the agglutinin titer of the serum from patients with brucellosis. Only two subjects without brucellosis had a titer over 1:40 by the Coombs method; one man had recently recovered from a dysentery caused by *Salmonella oranienburg* and the other had been given cholera vaccine. Several of the patients with brucellosis had recovered from the disease and their agglutinin titer was less than 1:160 by the tube dilution method; their Coombs antiglobulin titer, however, ranged as high as 1:640. The Coombs antiglobulin method nearly always removed the inhibition prozones.

Serial washings and addition of antiglobulin rabbit serum, which are an integral part of the Coombs procedure, did not contribute as much to the increase in agglutinin titer as did the centrifugation incident to the washing procedure. The most time-consuming and expensive features of the Coombs test were therefore eliminated. The serum dilutions need only be mixed with the Brucella antigen, incubated and centrifuged in order to obtain the highest agglutinin titer of the serum. Figure 5 compares the agglutinin titer of the serum of 102 subjects by the tube dilution and centrifugation methods. The results were practically the same as with the more cumbersome Coombs antiglobulin method. Only three of 47 "normal" subjects showed a Brucella agglutinin titer of more than 1:80 after centrifugation; one of these men was infected with *S. oranienburg* and two had received cholera vaccine. Generally the agglutinin titer of sera from patients with brucellosis was increased by four to eightfold by the centrifugation method. Centrifugation almost completely eliminated inhibition prozones and the "blocking" phenomenon. All the patients with proved brucellosis and most of those with proba-

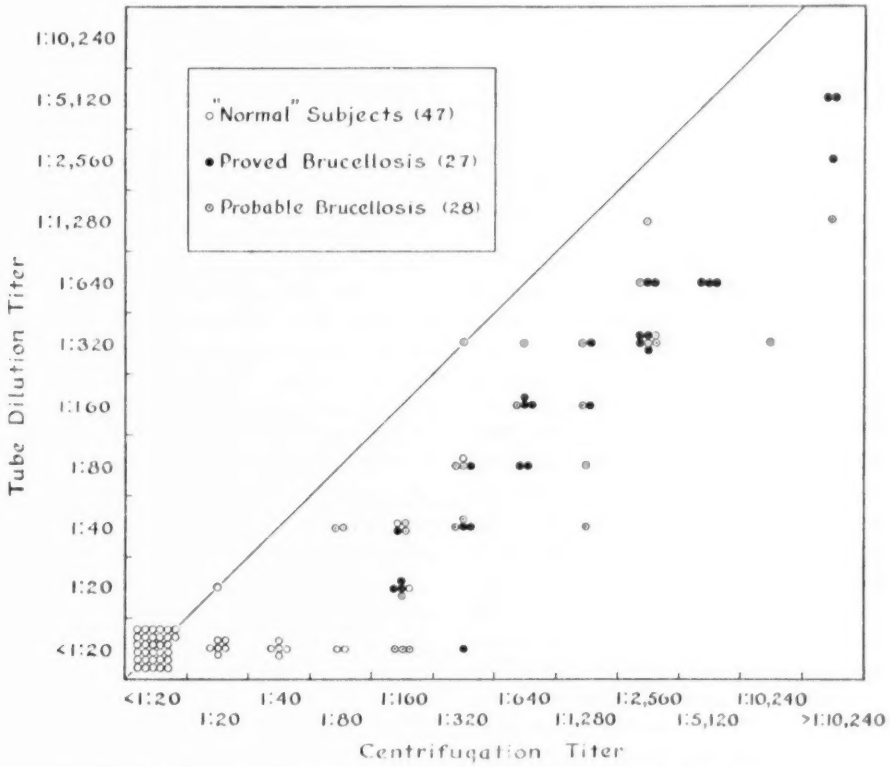

FIG. 5. THE BRUCELLA AGGLUTININ TITER IN 102 SUBJECTS BY THE TUBE DILUTION AND CENTRIFUGATION METHODS

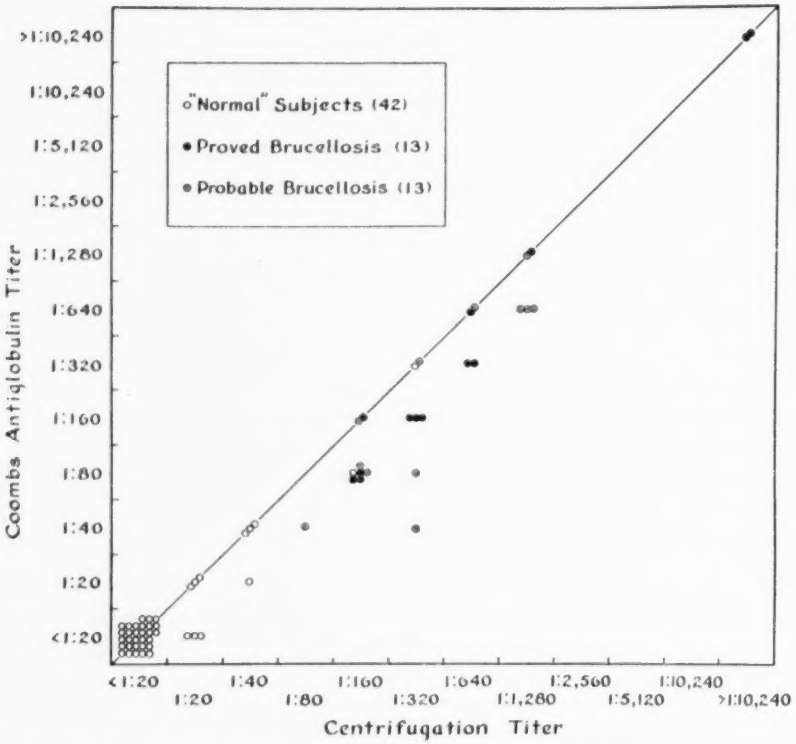

FIG. 6. BRUCELLA AGGLUTININS BY COOMBS ANTIGLOBULIN AND CENTRIFUGATION METHODS IN 68 SUBJECTS

ble brucellosis whose tube dilution agglutinin titer was less than 1:160 had contracted brucellosis many months earlier and were asymptomatic when tested.

Some variations of the centrifugation method were tried. Although washing of the antigen-antibody was not necessary, it removed background turbidity and made the agglutinated precipitate more compact. Interpretation of the degree of agglutination was thus made easier. The data given in Figures 5 and 6 were obtained by centrifugation of the incubated serum-antigen mixtures in a Sorvall angle centrifuge at 4,000 r.p.m. for 15 minutes. Almost identical results were obtained in comparative studies with six immune human sera using an International centrifuge (No. 2) at 2,500 r.p.m. for 30 minutes.

Close correlation between the results with the Coombs antiglobulin and the centrifugation methods was demonstrated by simultaneous examination of serum from 68 subjects (Figure 6). In some cases the centrifugation method gave an agglutinin titer two to four dilutions higher than did the Coombs method; in only one instance was the reverse true. Two "normal" individuals, previously mentioned, had agglutinin titers over 1:40 by both methods; one had a recent Salmonella dysentery and the other received cholera vaccine. The patients with brucellosis did not all have active disease when tested; several with low titers had recovered or were convalescing.

The results of paper chromatograms for Brucella antibody in the serum of 23 subjects are given in Table I. The normal individuals had negative chromatograms. All but one patient with brucellosis had "specific" Brucella antibody in his serum; the one exception occurred with a patient tested more than four years after he contracted the disease and long after he had recovered. The chromatogram showed "specific" antibody to be

TABLE I

Comparison of the Brucella agglutinin titer (tube dilution) and the paper chromatogram of the serum from 23 subjects

Status and no. of patients	Tube dilution Brucella agglutinin titer	Paper chromatogram*			
		Negative	Positive		
			Non-specific	Mixed	Specific
Normal—4	<1:20	4	0	0	0
Probable brucellosis—6		0	0	0	6
	<1:20				(1)
	1:80				(1)
	1:160				(1)
	1:640				(3)
Proved brucellosis—13		1	0	1	11
	1:20	(1)			(1)
	1:40				(2)
	1:80			(1)	
	1:320				(3)
	1:640				(4)
	1:1,280				(1)
	Totals =	5	0	1	17

* Method of Hess and Roepke (3).

present as early as 3 days and as late as 39 months after the onset of symptoms. The intensity of the fixation of antigen by "specific" antibody on the paper strip correlated only roughly with the Brucella agglutinin titer by the tube dilution method. A "non-specific" antibody (1+) was found in the serum of one patient with proved brucellosis due to *Br. abortus* during convalescence; his serum also contained "specific" antibody (4+) but the agglutinin titer was only 1:80.

Six normal adults were given cholera vaccine (Table II). The dose was 0.5 ml. subcutaneously followed by 1.0 ml. seven days later. Serum was obtained before each injection and weekly thereafter for six weeks. Brucella agglutinins were measured by the tube dilution and paper chromatographic methods. Four of the six showed a maximum Brucella agglutinin titer of 1:20 to 1:320 two weeks after the first dose of cholera vaccine. The titer did not exceed 1:80 in any subject at six weeks. Paper chromatograms showed that the Brucella agglutinins were composed of a mixture of "specific" and "non-specific" antibodies.

DISCUSSION

In our hands the tube dilution method has proved to be the most reliable test for Brucella agglutinins. It was the method used by Wright (7) when he first described the agglutination test for differentiating Malta fever from typhoid fever in 1897. It measures both the quantity and avidity of the serum antibody with a sensitivity and specificity offered by no other method. It would be an advance if the tube dilution method were to be generally accepted and used as the preferred method for Brucella agglutinin titration in laboratories throughout the world (8). Unfortunately a standard antigen is not universally available for the test. The B.A.I. antigen widely used by veterinarians in this country is an excellent antigen but it is not available for use with human sera except in a very few laboratories. Details of the method, including preparation of the antigen, were

TABLE II

Brucella agglutinin titer (tube dilution) and paper chromatogram of six normal subjects given cholera vaccine

Time	Control 0 week		0.5 ml. cholera vaccine subcutaneously	1 week		1.0 ml. cholera vaccine subcutaneously	2 weeks		3 weeks		6 weeks	
Subjects	Titer*	Chromatogram†		Titer	Chromatogram		Titer	Chromatogram	Titer	Chromatogram	Titer	Chromatogram
M. N.	0	0		0	0		0	M	0	N?	<20	M?
H. E.	0	0		<20	0		20	N	20	S	40	0
E. S.	0	0		0	N		0	M	0	M?	0	M?
R. W.	0	0		<20	0		<20	M	20	M	<20	M
R. F.	0	0		<20	0		160	M	160	M	80	M?
R. B.	0	0		80	M?		320	M	160	M	80	S?

* Reciprocal of final dilution giving 1+ or greater agglutination of *Br. abortus* antigen.
† Method of Hess and Roepke (3). S = "specific." N = "non-specific." M = mixed. 0 = negative.

worked out in detail by Fitch (4). Variations in antigens and method make it desirable to have standard immune sera available for comparisons among laboratories. The few comparative studies that have been published indicate that there is an appalling variation in results in different laboratories when the same sera are tested for Brucella agglutinins by various non-standard methods (9, 10).

The data presented in Figure 1 amply demonstrate that the tube dilution method is more sensitive and just as specific as the rapid macroscopic slide method of Huddleson despite previous conclusions to the contrary (11). The rapid slide method is less precise and less reproducible, often leading to considerable variation in results in different laboratories (9, 10). Furtheranore, certain lots of rapid slide antigen may be contaminated with agar and give rise to false positive reactions (12). Inhibition prozones were not observed with the rapid slide method yet were rarely troublesome with the tube dilution method. In fact the prozone obscured results of tube dilution agglutination tests in only one of several thousand such sera examined in this laboratory.

For purposes of rapid serologic diagnosis we have preferred the Castaneda rapid slide or screening test (1). As shown in Figure 2, this test has proved to be a suitably accurate measure of the presence and quantity of Brucella agglutinins in human sera. The test requires few materials and can be done in a few minutes at the patient's bedside. We have observed no nonspecific reactions and the test is seldom negative in patients with active brucellosis.

It has been stated by several authors that patients with active brucellosis frequently have no Brucella agglutinins in their serum (1, 13–19). This situation has been said to be particularly common in chronic brucellosis (20–23). One might explain its occurrence by localization or sequestration of the Brucella in an avascular focus (23). The studies of Griffitts (24) led some to believe that "blocking" or "incomplete" antibodies often might be found in brucellosis. Such antibodies have been found in certain sera by Schubardt (25), Renoux (26–28) and Jones and Wilson (29). Our own studies, however, indicate that such antibodies are seldom found in a titer which would significantly alter the agglutinin titer.

We have observed only one of more than 200 patients with brucellosis whose tube dilution agglutinin titer was less than 1:20 at any time during the active phase of his disease exclusive of the first two weeks. The one exception occurred in a veterinarian with recurrent, severe brucellosis caused by *Br. abortus*.[7] On several occasions his tube dilution agglutination test was reported to be "negative" by another reliable laboratory although he had a bacteremia. Earlier and subsequent agglutinins were demonstrated in a titer of 1:160 to 1:640 on several occasions. There usually was an inhibition prozone extending through a dilution of 1:160. "Blocking" antibodies were demonstrated in his serum at a dilution as high as 1:640. This undoubtedly accounted for the difficulties with agglutination tests in this case. The blocking activity of his serum was reduced, but not entirely inhibited either by heating to 56° C. for 30 minutes or by the addition of 5 per cent bovine albumin (Cohn's fraction V) or 5 per cent sodium chloride solution. The blocking activity of his and other sera was completely overcome by high-speed centrifugation of the serum-antigen mixtures. The blocking activity of his serum was also apparent with the macroscopic slide method (Huddleson).

The mechanism of the blocking phenomenon is unknown. The most reasonable explanation would seem to be the presence of Brucella antibody which is incomplete, lacking a chemical group necessary for spontaneous adherence of one sensitized particle of antigen to others, and yet possessing undiminished avidity for union with the antigen particle itself. The serum containing incomplete antibody, however, is able to cause agglutination of sensitized antigen particles after high-speed centrifugation. Hence, the defect in chemical grouping may be only a matter of slight change in configuration and not a complete lack of the necessary group(s).

That "blocking" antibodies are capable of uniting with their homologous antigen has been amply proved by the indirect Coombs method. The latter method has been recommended by Jones and Wilson (29) and by Wilson and Merrifield (30) for increasing the sensitivity of Brucella agglutination tests, eliminating prozones and overcoming

[7] We are indebted to Dr. Wesley W. Spink, University of Minnesota Hospitals, for this serum.

blocking antibodies. We have confirmed their findings with regard to the Coombs method (Figure 4). The Coombs method, however, is slow and requires very meticulous washing of serum-antigen mixtures to avoid false negative results. Also, the Coombs antiglobulin rabbit serum is too expensive for routine titration of Brucella agglutinins. Simple centrifugation accomplishes the same result as the Coombs method with greater simplicity and speed (Figures 5 and 6). Prozones and the blocking phenomenon were rarely seen after centrifugation. Indeed, centrifugation has been used by Feinberg and Wright (31) to allow brief incubation for one-half hour at 37° C. They concluded that with the tube dilution method it was better to incubate at 56° C. for 16 hours in order to lessen cross-reactions with non-specific agglutinins such as seen after tularemia and cholera vaccine. It has been our experience that heating sera obtained after cholera vaccination at 56° C. for 30 minutes does not significantly decrease their Brucella agglutinin titer (32). In our hands centrifugation did not permit brief incubation at 37° C.; agglutinin titers after 3 to 6 hours were generally lower than after 24 hours at 37° C. The possibility of brief incubation at 56° C. followed by centrifugation is now under investigation.

Brief experience with the paper chromatographic method of Hess and Roepke (3) indicates that although it is a sensitive method for detecting Brucella agglutinins in human sera the chromatograms are often difficult to interpret. Patients with brucellosis seldom show "non-specific" antibodies. However, normal subjects vaccinated with cholera vaccine tend to show a mixture of "specific" and "non-specific" agglutinins. The method does not seem to differentiate clearly Brucella agglutinins arising from cholera vaccination from those caused by Brucella infection. This is not surprising since Brucella possess an antigen closely related to the "H" antigen of *Vibrio comma* (33).

SUMMARY AND CONCLUSIONS

1. Comparative studies with over 100 human sera were made to evaluate methods for demonstrating Brucella agglutinins. The methods included the tube dilution, rapid macroscopic slide (Huddleson), rapid slide (Castaneda), indirect Coombs antiglobulin, "blocking," centrifugation and paper chromatographic methods.

2. The tube dilution method is more sensitive than Huddleson's rapid macroscopic slide test.

3. Castaneda's rapid slide test is useful as a quick bedside method to detect active human brucellosis. However, it gives only a crude index of the quantity of agglutinin.

4. The "blocking test" is seldom useful. Only one of more than 200 patients with brucellosis had sufficient "blocking" antibody to obscure the results of the tube dilution agglutination test.

5. The indirect Coombs antiglobulin method is a sensitive but tedious and expensive procedure for titrating Brucella agglutinins. It eliminates the problem of prozones and "blocking."

6. High-speed centrifugation after incubation of serum-antigen mixtures yields a sensitivity equal to or greater than that of the Coombs method with greater speed and simplicity.

7. Paper chromatography is a sensitive technique for detecting Brucella agglutinins in human sera but its usefulness in differentiating "specific" from "non-specific" agglutinins remains in doubt.

8. The tube dilution method, with a standard Brucella antigen and standard immune sera as controls, has been found to be the most useful, practical procedure for the demonstration of Brucella agglutinins.

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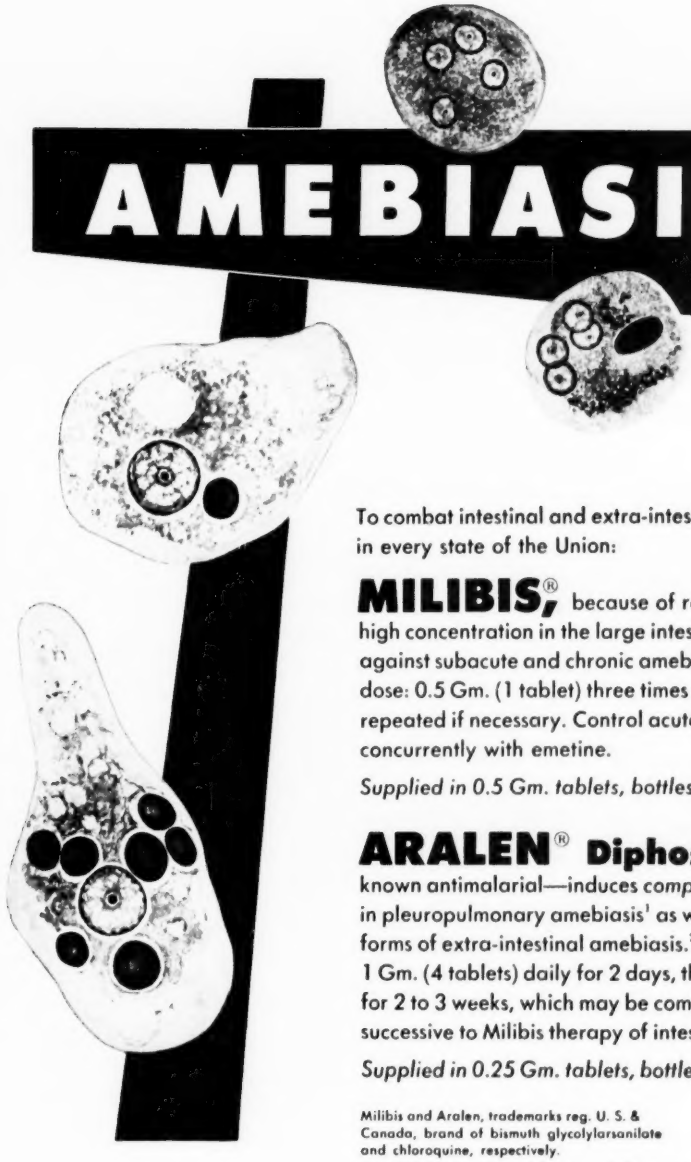

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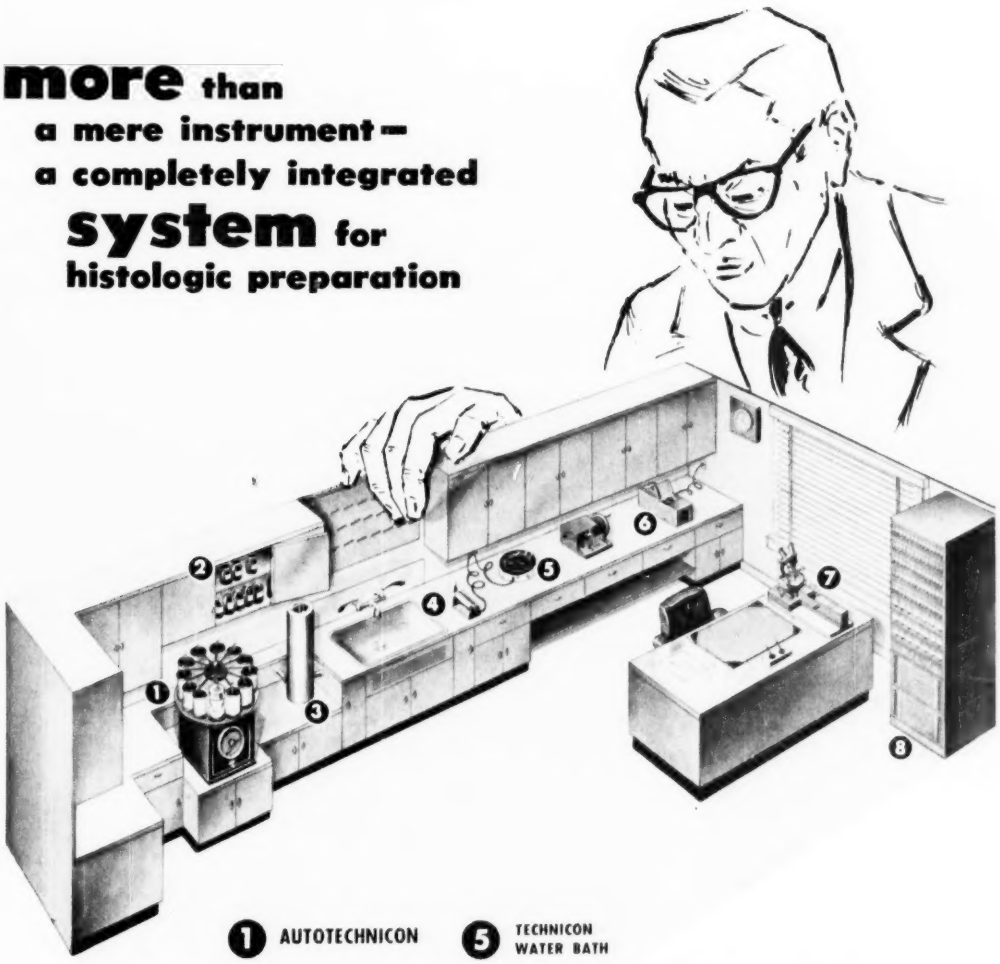

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